THE MORPHOLOGY OF THE PARASITE (HISTOPLASMA CAPSULATUM) AND THE LESIONS OF HISTOPLASMOSIS,[1] A FATAL DISEASE OF TROPICAL AMERICA.[2]

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Plates XIX–XXIII.

Soon after the occupation of the Canal Zone by Americans, in 1904, the medical officers of the Sanitary Department, on account of the numerous cases of splenomegaly met with among the Colombian natives, had their attention called by the work of Leishman[3] and Donovan[4] to a very fatal endemic disease of India, known as kala-azar. The clinical features of this disorder being splenomegaly, emaciation, cachexia, irregular pyrexia, leukopenia and the presence of an intracellular protozoon in the endothelium of the liver, spleen, lymph nodes, etc.

Manson[5] in one of his prophetic utterances has stated that kala-azar or an analogous disease might be found in America, and before this, Ronald Ross, during his visit to Panama in 1904, looked through the wards of Ancon Hospital in search of a case of splenomegaly associated with the kala-azar symptom-group, but none was found, although tropical splenomegaly is commonly seen in this region, particularly among the native Colombians; so common, indeed, that in the autopsy room it has been the custom to speak of examples of tropical splenomegaly met with here as "Colombian Spleens." The size of the Colombian spleen is due partly to an interstitial fibrosis and partly to lymphoid hyperplasia; etiologically,

[1] Darling, S. T., *Jour. American Med. Assn.*, 1906, xlvi, 1283; *Maryland Med. Jour.*, 1907, i, 125; *Arch. Int. Med.*, 1908, ii, 107.

[2] Received for publication April 2, 1909.

[3] Leishman, W. B., *Brit. Med. Jour.*, 1903, i, 1252; 1903, ii, 1376; 1904, i, 303.

[4] Donovan, C., *Brit. Med. Jour.*, 1903, ii, 79, 1401; *Indian Med. Gaz.*, 1904, xxxix, 321.

[5] Manson, P., Lectures on Tropical Diseases, Chicago, 1905.

it is not in any way related to kala-azar or to the disease described here.

During 1905–1906, while the writer, in search for the Leishman-Donovan body, was examining smears from the spleen, liver and rib-marrow of all cadavers at Ancon Hospital in which there was splenomegaly, he was rewarded by finding the microörganism associated with the lesions described here and elsewhere.

CLINICAL NOTES.

Two of the patients were Martinique negroes and the third was a Chinese who had lived on the Isthmus of Panama for 15 years. They had been ill several months and were much emaciated. All had had fever, but malarial parasites could be found in the blood of two of the patients neither before nor after death. A pronounced anemia was observed in all three cases. One patient had a leucocytic count of 2,200 per cu. mm. just before death. The most striking and characteristic feature, however, was the enlargement of the spleen. The temperature of one of the patients was observed for a period of three weeks when it was at first irregularly continuous and later regularly remittent about two degrees above normal. The irregularly continuous temperature may have been partly due to the malarial infection which was present at that time. The disorder is seen then to possess certain points of resemblance to kala-azar, viz., splenomegaly, irregular pyrexia, leukopenia, anemia, emaciation and chronicity.

Animal inoculations (guinea-pig) from material from Case III were negative and the microörganism disappeared from cultures and specimens of macerated tissue, probably on account of contaminating post-mortem bacteria which overgrew everything.

Probably on account of the vastly improved hygienic conditions in which the laborers live, this disease has disappeared for the present in the Canal Zone, but the writer believes that it will be found in other regions of Central and South America and the West Indies. It is to be noted that two of the cases were in Martinique negroes.

Cultural experiments and animal inoculations from material obtained during life from splenic and hepatic punctures will be of the greatest value in further attempts to assign a place to this microorganism in nature.

Judging from the extent of tissues invaded and destroyed by the parasite, Case III, that of the Chinese, is regarded as the advanced stage of the disease; while Case II, one of the Martiniquans, is thought to represent the earlier phase as the lesions were not so extensive and there were relatively fewer parasites, death in this case being due to an intercurrent broncho-pneumonia. Case I is intermediate in regard to the extent of tissue invaded, number of parasites and of organs attacked.

ANATOMICAL FINDINGS.

Post-mortem changes were delayed. The bodies were kept at a temperature of 74 to 92° F., yet the decomposition usually seen in bacterial infections was absent. Emaciation was well marked, though not extreme.

Skin.—A few recent papules, 8 mm. in diameter, were observed in the skin of the right arm in Case III.

Subcutaneous Tissue.—Slightly edematous in Case III.

Musculature.—Atrophy of all the muscles, particularly the sartorius muscle in Case III.

Pleuræ.—There were a great many ecchymoses, 8 mm. in diameter, scattered beneath the visceral pleura of the lungs in Case I, which corresponded to underlying hyaline nodules, 2 to 4 mm. in diameter. Fewer of these hemorrhages were seen in Case III and they corresponded to the smaller number of pseudo-granulomata noted in this case.

Lungs.—In Case I, the lungs presented a picture very much like that of miliary tuberculosis, for on section the lungs were dotted everywhere with dense pale gray hyaline granulomata, 2 to 3 mm. in diameter. The granulomata were not so numerous or so closely packed as in miliary tuberculosis and they were surrounded by narrow margins of hemorrhage. In Case III the granulomata were in some instances elongated and larger, as though several smaller ones had coalesced. These too were surrounded by an area of hemorrhage. These granulomata were always of a peculiar grayish hyaline tint and were quite dense and smooth on section. No granulomata were detected in the lungs in Case II, which showed the mildest or lightest infection, but there was a terminal broncho-pneumonia.

Peribronchial Lymphnodes.—These were enlarged and contained black pigment in all cases, and in Case I there were a few small fibro-caseous tubercles.

Heart.—This organ was small in all cases and was proportionate to the atrophy of the other muscles.

Liver.—In the advanced stage of the disease as shown in Case III, the liver presented a very unusual appearance, due to extensive necrosis and cirrhosis. The organ was enlarged and its capsule smooth, while scattered throughout there were many areas of necrosis, grayish yellow in color, the larger ones

frequently being arborescent in shape. The arborescent areas involved the portal radicals which they followed; their centers were gray and their peripheries yellow. The arborescent twigs had four to six limbs. They were not so numerous near the falciform ligament as elsewhere.

In the earlier stage of the disease as represented by Case II, the necroses were much smaller, fewer in number and were not visible macroscopically.

Portal Lymphnodes.—These nodes in Case III were enlarged, pale yellow, slightly edematous and friable.

Spleen.—The spleen was very markedly enlarged in each case, varying from about 450 to 750 grams in weight. It was more suggestive of the appearance and consistence of the organ in spleno-myelogenous leukemia than of anything else and was distinctly different from any other type of spleen met with in Panama. The capsule was smooth and tense. The entire organ was rigid and not flexuous to the slightest degree. The pulp was firm in consistence yet friable. The pulp was dark red in color and the Malpighian corpuscles were visible in Case I and indistinct in Case III.

The Lymphnodes at the Hilum of the Spleen.—These were enlarged and pale.

Pancreas, Kidneys and Adrenals.—There was nothing of note.

Intestines.—Case III presented a number of very curious areas of pigmentary, hyperplasia and ulceration in the jejunum, ileum and colon; there were about fifty in the former and twenty-four in the colon. The earliest lesion appeared to be that of hyperplasia or the formation of a small nodule in the mucous and submucous coats; these nodules were 5 to 6 mm. in diameter, and later, became reddened and infiltrated around their peripheries; necrosis and circular ulceration appeared, sometimes with hemorrhage from the ulcer. The last phase was that of a puckered, pigmented, black scar about 6 or 8 mm. in diameter. The size, distribution and peculiarly black pigmentation of these ulcers with the black pigmentation of the neighboring visceral and parietal peritoneum made them of striking appearance and unusual interest. The postperitoneal lymphnodes were enlarged and pale, while the mesenteric lymphnodes in this case were not appreciably enlarged.

In Case I there were a few superficial circular ulcers in the cecum and ileum, and some hyperplasia of the mesenteric lymphnodes. The bone-marrow, brain and membranes, accessory nasal sinuses, joints, blood vessels and genitalia, showed no gross lesions referable to the infection.

THE PARASITE.

The microörganism was present in enormous numbers in the tissues, generally intracellular in large mononuclear endothelial cells in the liver, spleen, lymph nodes, submucous nodules in the ileum and colon, and in the hyaline nodules in the lungs.

Smears from the lung nodules contained great numbers of large cells resembling alveolar epithelial cells enclosing from twelve to three hundred parasites (Fig. 3).

Smears from the liver and spleen showed almost the same picture

(Fig. 9). The individual cell infection was not so great; that is, there were not so many parasites in a cell but there was a very large number of free parasites.

The parasite is best seen in very thin smears from invaded tissue such as spleen, liver and lung nodules. It is round or oval in outline and in size from 1 to 4 μ through its greatest diameter; commonly this diameter is 3 μ. The larger forms are more frequently oval.

When stained by some of the polychrome blue and eosin stains—Leishman's or Hasting's stains—the entire structure is well displayed and one is struck immediately by the various forms and positions taken by the chromatoid substance or nucleus. This substance is sometimes globular or oval and is placed at one end of the parasite, or it may be distributed half way along the periphery. There may be little dot or dash-shaped off-shoots into the interior from an irregular mass or ring of chromatin at one end of a parasite. Hardly any two parasites look alike. Besides the chromatoid substance there is some blue staining basophilic substance making up the remainder of the parasite and filling out the oval or round outline, except for an achromatic zone generally placed near the center (Fig. 3).

The parasite is surrounded by a refractile capsule, one-sixth of the diameter of the parasite in width, which ordinarily does not stain. In hematoxylin stained smears it is possible, occasionally, to demonstrate a single minute dot in this refractile capsule. A number of the parasites contain one or more small chromatoid granules located in the center or on the periphery. While these chromatoid granules are generally solitary, at times two or more may be counted. A few large parasites have several chromatoid dots. These granules are frequently situated in an achromatic zone or upon the margin either near or within the basophilic substance or even in the refractile capsule.

The chromatoid granules are generally spherical, very rarely elongated, and occasionally two granules in apposition simulate a rod.

The achromatic zones resemble the achromatic zones seen in malarial rings, filaria embryos, trypanosomes and various protozoa.

The parasite appeared to divide by fission into two equal or unequal elements (Fig. 2). One apparently was dividing into four equal elements, while several parasites with chromatoid dots

scattered through their substance appeared as presegmenting bodies ready to divide into five or six elements (Case I). Occasionally a smaller parasite may be seen close beside a larger one as though separating from it, the smaller one being about 1 μ in diameter.

The lack of uniformity to the disposition of the chromatic and achromatic substance in the parasite gives a very varied picture, depending partly on the part presented to the observer.

In Case I this was particularly noticeable in smears from the lung nodule. Forms suggesting the appearance of familiar objects, such as a conch shell, bullet, shuttle, the eye and palpebral fissure were seen. The arrangement of the chromatoid substance along the periphery of the parasite in certain specimens was strikingly imitative of a mammalian embryo in " fetal attitude."

Three flagellated forms were seen in a smear from the lung in Case I, and two from a spleen smear in Case III. The distal extremity of the flagella contained a rod of chromatin placed at right angles to the flagellum, simulating the relation of centrosome to chromatin filament in *Trypanosoma lewisi.* The flagella were single, short and thick, without chromatin filaments and were enclosed by the refractile capsule of the body of the parasite.

In smears from the lungs the parasite was almost always intracellular (Fig. 3). The cells enclosing the parasite were large mononuclear cells resembling large endothelial or alveolar epithelial cells. However, as none of the invaded cells in lung smears contained dust particles they may have all been endothelial cells. The invaded cells contained from eight or ten to one hundred or more parasites of slightly varying sizes or with a larger parasite surrounded by a clump of smaller ones. One unbroken cell in a lung smear contained more than three hundred parasites which had invaded the nucleus as well as the cytoplasm. This infected cell occupied one-third the diameter of the one-twelfth oil immersion field. Smears from the spleen and rib-marrow contained fewer parasites, although still numerous, and they were often extracellular. The nucleus of the cell appeared now and then to have been invaded. Wherever found the parasite was always enclosed in a refractile capsule. Red blood corpuscles were never invaded.

One of the most striking characteristics of the parasite is the lack

of uniformity to the distribution and arrangement of the chromatic and achromatic substance.

The parasite always takes polychrome blue stains like a living or recent microörganism.

Twenty-four hours after the death of the host, the chromatin and basophilic substances stain beautifully, while under similar conditions the malarial parasite would not stain so well and the chromatin dot might not stain at all.

In rib-marrow smears it was noticed that the microörganism required more prolonged contact with the stain to obtain good results. This was thought to be due to the impenetrability of the refractile capsule.

HISTOLOGICAL EXAMINATION.

Tissue was fixed in Zenker's solution and in formol, and paraffine sections were stained by eosin and hematoxylin, eosin and methylene blue, by Marchi's, Van Giesen's, and by Weigert's fibrin method. A few celloidin sections were stained by eosin and a polychrome methylene blue, differentiated with anilin oil. This proved to be an excellent stain for the parasite in tissue. Gentian violet also stained the parasite intensely.

In the description of the microscopic appearance of the tissue each case will be considered separately.

Taking up Case II first, in which, while there was a considerable degree of splenomegaly, the lesions in the liver were not at all advanced and the case altogether may be regarded as an early stage of the disease; this will be followed by a description of the lesions in Case I, in which the disease was well advanced in the lungs, liver and spleen. Finally Case III, which represents a very advanced stage of the disease with the splenomegaly, extensive liver necroses, intestinal ulceration and pulmonary pseudo-granulomata will be described.

CASE II. G. B. HOSPITAL NUMBER 9141.

Liver.—The columns of liver cells everywhere appear flattened and attenuated and the capillaries correspondingly increased in diameters. Here and there are areas of necrosis, in size up to .2

mm. in diameter, scattered throughout the lobule, apparently arranged irrespective of zone. Some of these areas of necrosis extended outward from portal spaces; others were in the intermediate zone, while others could not be oriented on account of the obscuration of the zone limits. In these areas of necrosis the debris has been almost completely removed, but here and there may be seen a small patch of coagulative necrosis or granular debris, yet on the whole the necrotic area has been replaced by young connective tissue cells and also by exudative cells similar to those seen in the kidney. These areas frequently contain one or more very large cells, 24 to 30 μ in diameter, distended by parasites. One of these areas of necrosis contained a typical giant cell with a mural arrangement of its nuclei on one side. The giant cell was not a part of a tubercle and around it were no round cells, epithelioid cells nor nuclear fragmentation. This giant cell was in the intermediate zone of a lobule. Following along the capillaries there are seen collections of parasites in endothelial cells. These endothelial cells have oval eccentric nuclei and their cytoplasm is invaded to a greater or less extent by parasites. The parasites are enclosed in a colorless chamber which in the lightly infected cells is generally much larger than that necessary to enclose them, while in the more heavily infected endothelial cells, this colorless chamber is obscured by the great numbers of contained parasites. The periphery of the infected endothelial cell is almost fibrous in character and remains intact when the nucleus and cell contents have disappeared. Many of these infected endothelial cells contain malarial pigment as well. The malarial pigment, however, appears to be near the periphery of the cell and not mixed in with the parasites. This proves the endothelial nature of the parasite-bearing cell. From an examination of this section, the earliest lesion is apparently that of the invasion of the endothelial cell. The portal veins in some of the medium sized portal spaces contain a good deal of debris, also a few endothelial cells containing parasites and malarial pigment, or, occasionally, a leucocyte or red blood cell. In the more lightly infected endothelial cells, the parasites are not always of the same size; for instance, there may be one large oval parasite and clustered around it six or seven parasites of decreasing size. No free parasites could be detected in the section.

The capillaries contain, besides, red blood cells staining variously with eosin, a number of which are washed out and their hemoglobin scattered here and there in coarse granules.

Spleen.—The capsule is 80 μ in thickness and is not appreciably thickened. There is slight atrophy of the Malpighian corpuscles. The splenic spaces of the pulp are distended by red blood cells and mononuclear cells and a few polymorphonuclear leucocytes. Scattered here and there are large mononuclear cells with small round or oval nuclei, the cytoplasm being distended by parasites. Some of these lie free in the distended splenic spaces, while others are attached to the wall of the splenic space and are endothelial in character. A number of them contain malarial pigment and red blood cells, and a few contain parasites, red blood cells and malarial pigment. There is on an average two or three infected cells to the oil immersion field. Here and there are a few parasites in groups of five or ten apparently free, but by far the greatest number are intracellular. The infected cells contain anywhere from two or three to fifty or more parasites. The connective tissue reticulum does not appear to be thickened and the size of the spleen is due to the engorgement of the splenic spaces and the invasion of the endothelial cells by parasites.

Lung.—The capillaries are tortuous and engorged with red blood cells. The alveoli contain red blood cells and polymorphonuclear leucocytes in about equal numbers and a few desquamated alveolar epithelial cells. Quite a number of the alveoli contain coagulated albumin and a few contain some small clumps staining like fibrin. The lumen of the small bronchi contain red blood cells, polymorphonuclear leucocytes and a few desquamated alveolar epithelial cells. No parasites could be detected.

Portal Lymph Node.—The node shows a diffuse hyperplasia of small and large lymphoid cells, particularly the latter. The connective tissue of the germinal centers in the follicles of the cortex shows extensive hyaline degeneration. The lymph sinuses in the cortex and medulla contain great numbers of large mononuclear cells about 20 μ in diameter, with round, oval, or horseshoe nuclei 4 to 7 μ in diameter. These large cells show frequently double nuclei and mitoses and are undoubtedly being reproduced rapidly. They are

ravenously phagocytic and have engulfed malarial pigment, red blood cells and a very few of them contain colorless chambers enclosing parasites, while a few others have picked up two or three parasites, as yet without the development of the achromatic chamber. The structure of the node is loose rather than dense and the small lymphocytes, which make up the bulk of the cellular elements in the dense lymphoid tissue, have deeply staining nuclei and are from 7 to 9μ in diameter, the nucleus being from 4 to 7μ in diameter.

Kidney.—The most striking feature is the increase in the intertubular connective tissue; both in the cortex and medulla, the tubules are separated by collections of cells, most of them of the plasma cell or polyblast type, round or oval in outline with eosin staining cytoplasm and rather large, generally eccentric nuclei. Some of them have double nuclei and a few show mitoses. In addition to these cells, which very strongly impress one as exudative, there are quite a number of polymorphonuclear leucocytes in the same localities and, occasionally, in the lumen of the convoluted tubules. Here and there are small hemorrhages into the intertubular connective tissue. The convoluted tubules are, generally, dilated and their epithelium, subcuboidal and flattened. The nuclei of the cells stain well, though not deeply. There are occasional examples of diapedesis of mononuclear and polymorphonuclear leucocytes through the epithelium into the lumen. The latter contains some hyaline casts, granular detritus, a few leucocytic plugs and some macerated desquamated epithelial cells. The glomeruli are pretty tightly enclosed by their capsules and are, apparently, normal. A few convoluted tubules show colloid degeneration of their epithelium. No parasites were detected.

Adrenal, Heart, Pancreas.—No parasites could be detected and the organs present nothing of note.

CASE I. C. D. HOSPITAL NUMBER, 7715.

Liver.—The entire section is dotted over with pale areas of necrosis in size all the way up to .8 mm. in diameter; many of them are from .24 to .8 mm. in diameter. These pale areas of necrosis involve all zones of the lobule, several of them involving two or

more lobules. Most frequently they seem to extend outwards from a portal space. In these areas there is more or less complete destruction and removal of the hepatic cells, leaving a reticulum composed of the stroma of the liver in the spaces of which are the granular and fatty remains of some of the hepatic cells, together with a great number of parasites enclosed within the endothelial cells of the part. In some of the areas there has been a slight proliferation of round cells and this appeared to be oftenest seen near a portal space. In most cases the disappearance of liver cells followed upon a granular and fatty degeneration, but in a few instances the necrosis has been coagulative in character with nuclear fragmentation. The necrosis at times fades gradually into areas of normal hepatic cells between which there are half-way stages of necrosis, while at other times an area of necrosis is outlined in large part by normal hepatic cells. In the uninvaded areas the liver cells take the stain beautifully and their outlines are quite distinct. There is no inflammatory reaction to be seen anywhere, polymorphonuclear leucocytes being very scarce. Of the leucocytes in the liver capillaries, the mononuclear elements seem to predominate. The invaded endothelial cells are the same size as those seen in the spleen. Extending outward from the areas of necrosis along the portal capillaries into regions of normal liver substance there is an almost unbroken line of endothelial cells invaded by parasites.

Spleen. –There is some atrophy of the Malpighian corpuscles. The splenic spaces are distented by red blood cells and some large and small mononuclear cells. Quite a number of these large mononuclear cells have engulfed red blood cells. The reticulum of the splenic spaces is not thickened. Here and there are large mononuclear cells free in a sinus or attached to its wall containing one or two dozen parasites and there are a few free parasites.

Lung.—Section of hyaline nodule (Fig. 4): the nodule appears to be made up of the collapsed and proliferated alveolar walls, although the capillaries of the alveoli can be made out with distinctness only near the periphery. The nodule is reticulated and scattered through the reticulum are round, oval and polygonal cells from 6 to 15 μ in diameter, their nuclei being from 4 to 5 μ in diameter. These cells are probably exudative in origin and re-

semble the cells described in the intertubular connective tissue spaces in the kidney of Case II. Side by side with these cells are a few red blood cells and the much larger cells resembling endothelial cells or alveolar epithelial cells with the achromatic chambers containing parasites. While most of the parasites are intracellular, a few are free; polymorphonuclear leucocytes are conspicuously absent.

Beyond the periphery of the nodule the capillaries are tortuous and are distented by red blood cells. The alveoli are empty. The periphery of the nodule contains some alveoli enclosing red blood cells and a few large mononuclear cells with single or double nuclei containing large numbers of parasites. The parasites are sometimes within one large chamber in the cell, or there may be several smaller chambers, each one containing two or three to a dozen parasites. These mononuclear cells are from 20 to 30 μ in diameter.

Lymph Node from Hilum of Spleen.—This node, particularly in the loose lymphoid tissue and in a few cortical follicles, has undergone a granular and, in places, coagulative necrosis. The dense lymphoid tissue is not so much involved but the loose lymphoid tissue everywhere shows cellular necrosis. The lymph sinuses contain large numbers of large mononuclear cells distended by parasites (Fig. 5), apparently resulting from the necrosis of the mononuclear cells containing them. Several of the cortical follicles and medullary cords show extensive necrosis and nuclear fragmentation, the central portions of which are coagulative in character. These areas contain many parasites. The lesions in this lymph node represent a more advanced stage than in those described from the lymph node in Case II.

Peribronchial Lymphnode.—This node contains several old encapsulated fibro-caseous tubercles and one giant cell. The reticulum and capsule of the node were greatly thickened in places. A lymph vessel beneath the capsule contained mononuclear cells invaded by parasites.

CASE III. FU. MAS. HOSPITAL NUMBER, 17184.

Liver.—The liver presents an extraordinary appearance on account of the great destruction of hepatic cells and the invasion by parasites. There are large areas of necrosis .8 to 1.2 mm. in

diameter, irregular in outline, with extensions into the more nearly normal liver substance. The oldest areas appear to be those in and extending outward from the portal spaces, in fact every portal space is involved in an area of necrosis or its partial replacement by connective tissue and an invasion by parasites, while on the other hand several central and sub-lobular veins are surrounded by normal liver cells, but the process of destruction is so extensive that every zone sooner or later becomes involved. Fully two-thirds of the section is represented by areas of necrosis and the partial replacement by connective tissue and parasites. The destructive process in part consists of a granular and fatty degeneration of the hepatic cells and in part of a reticulated coagulated necrosis always associated by the presence of myriads of clustered groups of parasites (Fig. 8). As the hepatic cells are destroyed their places are taken by collections of parasites, which are sometimes free, but oftener enclosed in large mononuclear cells representing the endothelial cells of the capillaries. These enormously distended endothelial cells so fill up the spaces formerly occupied by the hepatic cells that there is no gross contraction or distortion of the framework (Fig. 7). In the older areas of necrosis the connective tissue stroma had become fibrous with relatively few cells, and the lymph spaces of this reticulum are distended by intracellular and extracellular collections of parasites. In the areas of coagulative necrosis there are numerous free parasites, either singly, or in twos, or threes. Throughout the section there are large patches involving portions of three or four lobules in which the hepatic cells are flattened and atrophied. The hepatic cells here contain much bile pigment and small fat droplets. The capillaries are engorged with red blood cells and a number of the endothelial cells are distended by parasites. One of the sub-lobular veins which is surrounded by an area of necrosis contains a large cell attached to its wall projecting out into the lumen. This cell is 24 by 27 μ in area, its nucleus is oval 4½ by 6 μ in area and is eccentric. This cell contains a clear central chamber 25 μ in diameter filled with parasites of varying sizes. This cell is almost free from its attachment, three-fourths of its periphery bulging out into the lumen.

The heaviest invasion by parasites was in the connective tissue

and endothelial cells, which had replaced the areas of necrosis; these areas correspond to the hyaline arborescence noted at autopsy.

The capsule of the liver was thickened and frequently communicated with the infected underlying areas of necrosis, but the capsule showed no invasion by parasites. The epithelium of the bile capillaries was not invaded by parasites.

Spleen.—The capsule is uniformly 96 μ in thickness. The Malphigian corpuscles show considerable atrophy of their lymphoid elements and there is an increase of fibrous tissue around the artery and in the stroma of the corpuscle. The splenic spaces are greatly distended by red blood cells. The connective tissue frame-work of the spleen is not thickened and the great bulk of the spleen is due to the uniform distention of the splenic spaces by red blood cells. One of the most striking features in the section is the numerous collections of parasites which are dotted everywhere throughout the spleen, there being a dozen or more collections to every oil immersion field. In the splenic spaces they are frequently contained within large mononuclear cells, which are either free in the sinus or attached to its wall (Fig. 10); the greater number of parasites are in the latter locations. There are many parasites in groups of two or three dozen free and intracellular throughout the reticulum of the loose adenoid tissue. The splenic spaces are relatively free from lymphoid cells. The cells which are present are large mononuclear in type with round or horseshoe nuclei and there are a few polymorphonuclear leucocytes. The Malphigian corpuscles are extensively invaded by parasites which appear to be either free in small groups or enclosed within large mononuclear cells.

Section of Lung Including a Hyaline Nodule.—The hyaline nodule resembles that described in Case I, but the reticulum seems to be a little more fibrous in character and contains relatively fewer cells. The central portions of the nodule contain relatively few parasites but nearer the periphery there are many large endothelial-like cells which contain two or three hundred parasites.

Many of the alveoli are distended, empty and emphysematous, while others are filled with serum or red blood cells. Just outside the margin of the hyaline nodule the alveoli are emphysematous and filled with red blood cells and frequently with three or four or

more large cells with mononuclear eccentric nuclei and achromatic chambers filled with parasites. These infected cells are from 18 to 30 μ in diameter with round or slightly oval nuclei 5 μ in diameter.

Bronchiole.—This section of one of the larger bronchioles is normal and no parasites could be detected anywhere.

Peribronchial Lymphnode.—This node showed several large areas replaced by dense fibrous tissue in the periphery of which there was a good deal of black pigment. Here and there where the lymphoid tissue still remained there were large cells sometimes appearing to form part of the reticulum and there were also endothelial cells filled with parasites bulging out into the lymph sinuses.

Ulcer of Ileum.—The peritoneum and muscle wall are normal but the mucous membrane is pushed away from the muscle wall by an oval mass, 1 by 2.8 mm. in area, of large round and oval cells, most of which have a large amount of eosin-staining cytoplasm, more or less replaced by parasites. The periphery of this nodule nearest the lumen of the intestine is denuded of mucous membrane almost to the depth of the muscularis mucosa. Under the high power there is seen to be an extraordinary invasion of certain new cells in the nodule by parasites. The invaded cells closely resemble the invaded endothelial cells of the liver. They are 15 to 30 μ in diameter and their round or oval nucleus, which is generally pushed to one side, is 4 to 6 μ in diameter. Right beside these cells are other smaller cells from 6 to 10 μ in diameter with eccentric small round, oval, or mitotic nuclei, which are apparently proliferating in the lymph spaces of the nodule and which have more deeply staining cytoplasm. The nucleus of the invaded cell is either eccentric or has been pushed to one side and the enclosed parasites are clustered in an achromatic chamber similar to infected cells in other viscera. This achromatic chamber has no definite membrane and is surrounded by the eosin-staining cytoplasm of the cell. Some of the invaded cells did not show this achromatic chamber, but merely a cluster of parasites imbedded in the cytoplasm of the cell (Fig. 11). In this section the blood vessels were not involved, but the lymph spaces everywhere in the nodule showed an invasion of their lining endothelium by parasites. While most of the microorganisms were intracellular, quite a large number was apparently

free. The epithelium of the glands of Lieberkühn was everywhere free from invasion by parasites, yet the inter-glandular connective tissue stroma up to the basement membrane was crowded with them (Fig. 6). The denuded surface of the ulcer was rich in infected cells and there were detached cells lying free on the surface. The morphology of these free parasites on the surface was the same as those imbedded in tissue—no flagellated forms could be detected. This section shows a more intense invasion by the parasite than that of any other tissue and the parasites apparently had spread along the lymph spaces.

Kidney.—There was some edema. The epithelium of the convoluted tubules was swollen and in places desquamated and cloudy. Most of the nuclei failed to take the stain well. Many of the tubules contained granular debris, desquamated epithelium and occasionally a hyaline cast. There was a slight increase in the intertubular connective tissue and in Bowman's capsule. The space between the glomerulus and Bowman's capsule frequently contained a large amount of coagulated albumin. No parasites could be detected.

CONCLUSIONS.

Histoplasmosis is a fatal infectious disease of tropical America resembling kala-azar of India. It is characterized clinically by splenomegaly, emaciation, irregular pyrexia, leucopenia and anemia. The pathological features are the invasion of endothelial cells in the smaller lymph and blood vessels and capillaries by enormous numbers of a small encapsulated microörganism (*Histoplasma capsulatum*) causing necroses of the liver with cirrhosis, splenomegaly, psudo-granulomata of the lungs, small and large intestines, with ulceration of the latter, and necrosis of lymph nodes draining the injected viscera.

The disease is caused by a small round or oval microörganism 1 to 4 μ in diameter possessing a polymorphous, chromatin nucleus, basophilic cytoplasm and achromatic spaces all enclosed within an achromatic refractile capsule.

The microörganism differs from the Leishman-Donovan body of kala-azar in the form and arrangement of its chromatin nucleus, and in not possessing a chromatin rod.

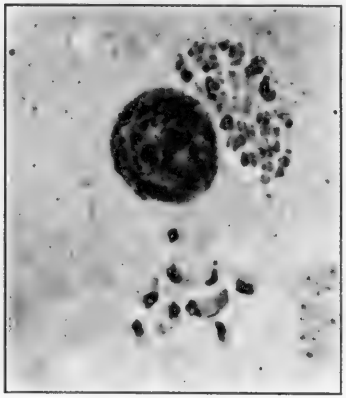
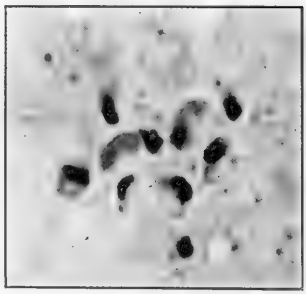


FIG. 1.



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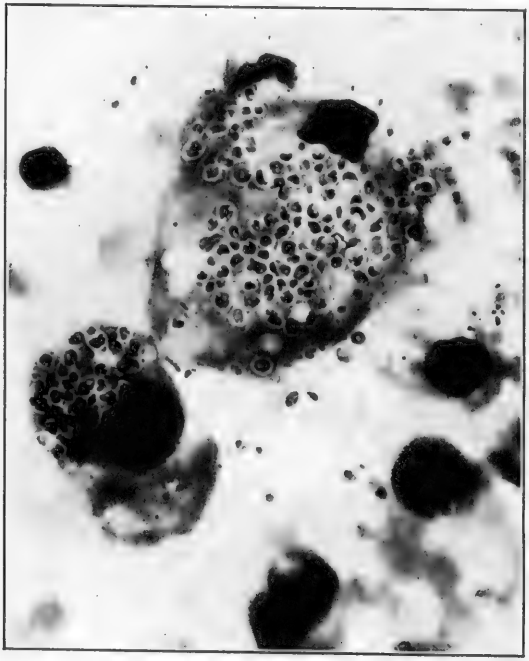

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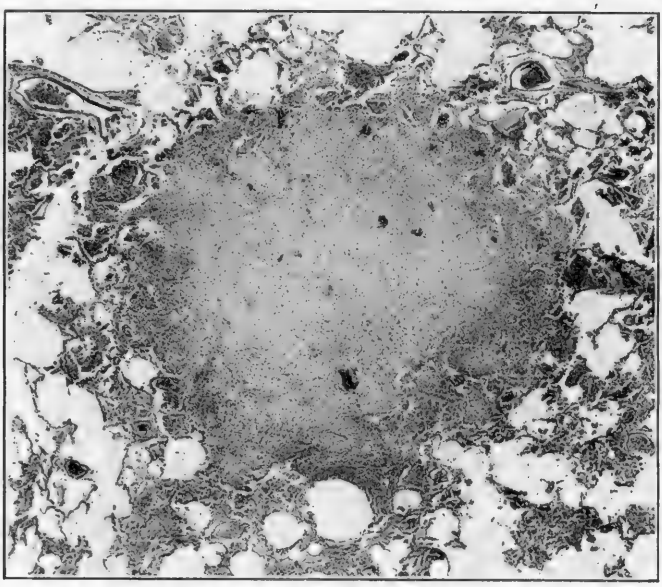

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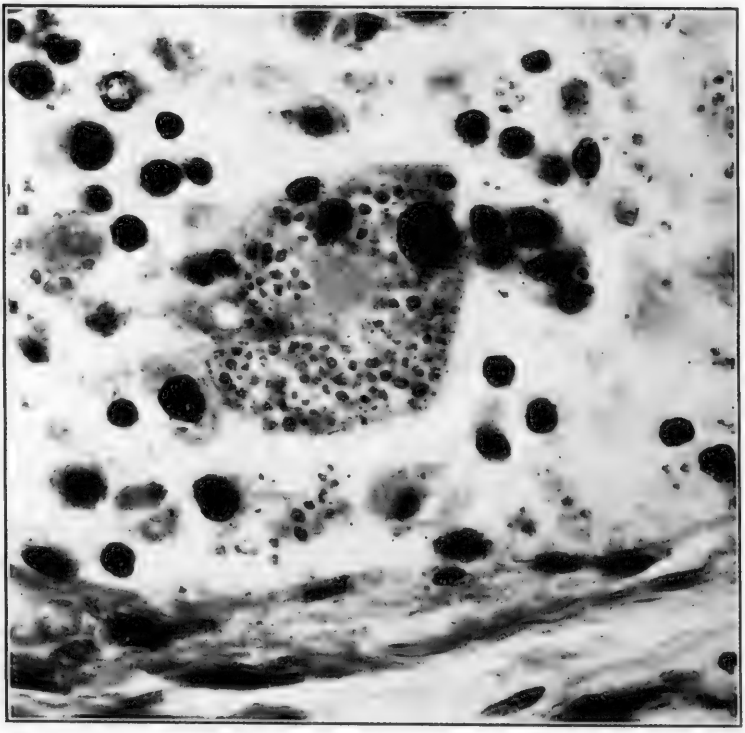

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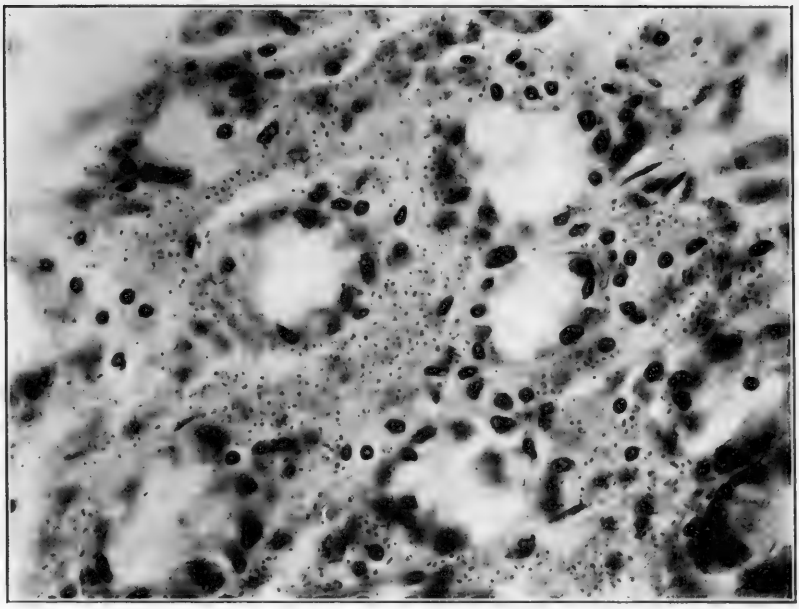

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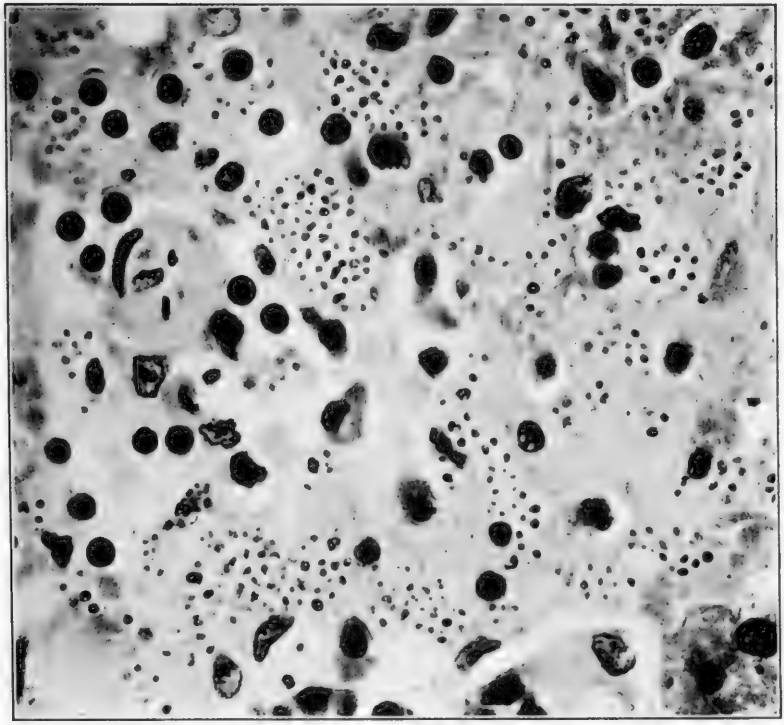

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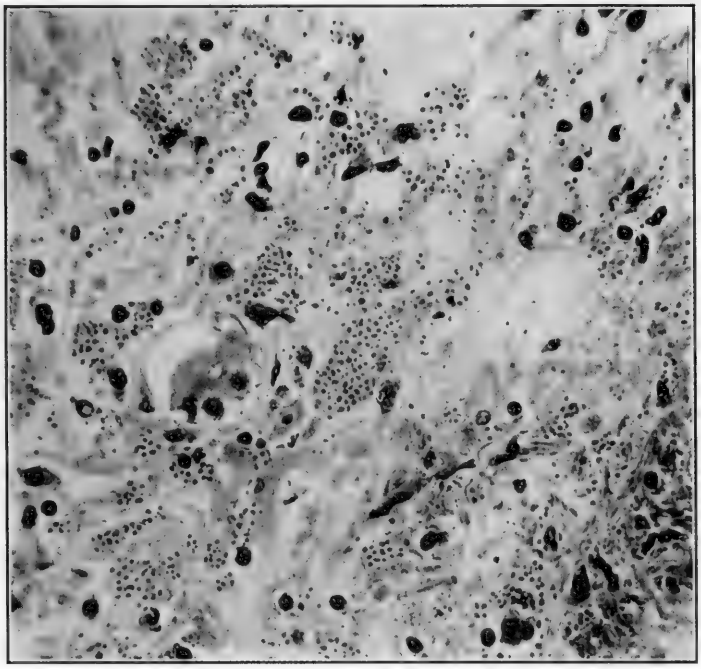

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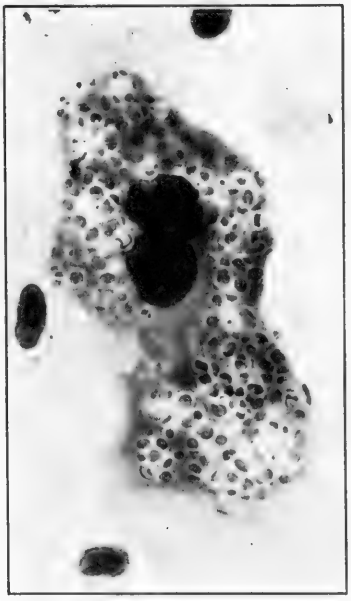

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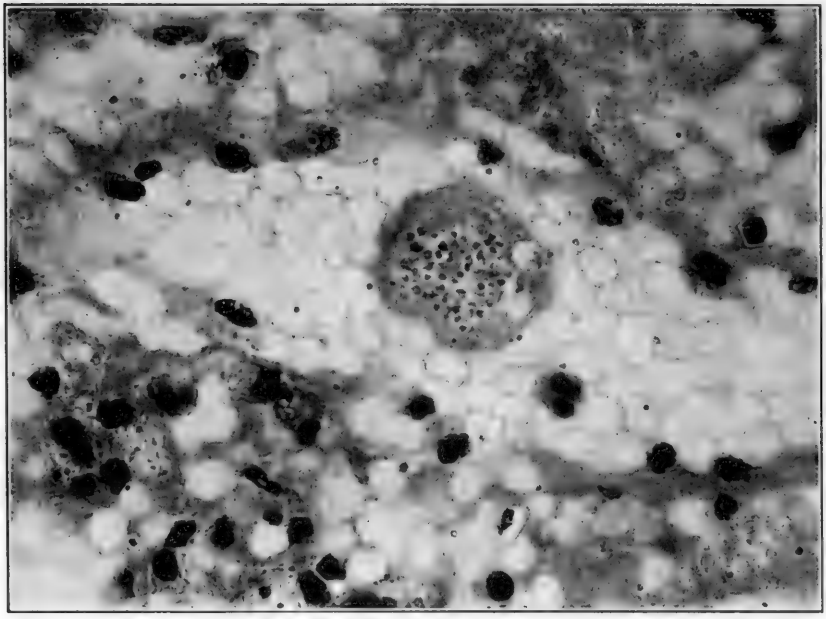

FIG. 10.

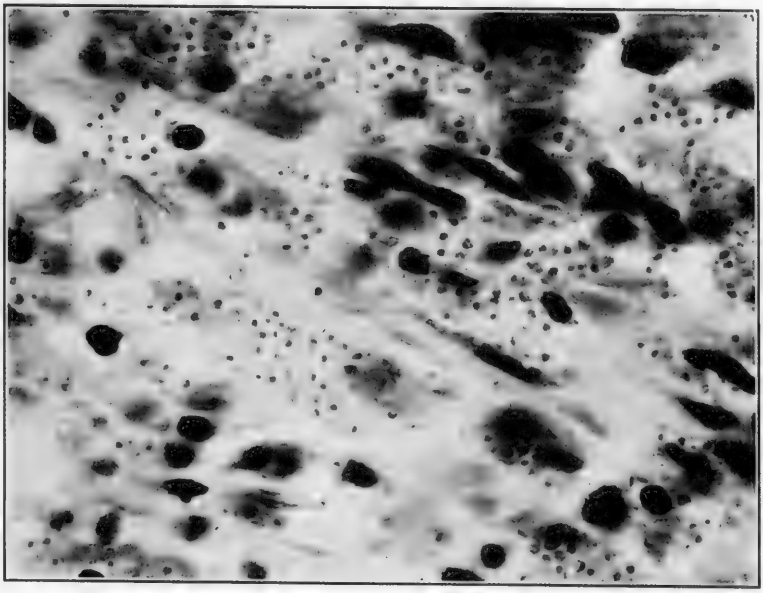

FIG. 11.

The distribution of the parasite in the body is by the invasion of contiguous endothelial cells of the smaller blood and lymph vessels and capillaries, and the infection of distant regions by the dislodgement of infected endothelial cells and their transportation thither by the blood or lymph stream. Thus the skin, intestinal and pulmonary nodules may be due to secondary distribution of the parasite.

The microörganism apparently lives for a considerable period of time in the tissues, because in the older areas of necrosis there are myriads of parasites all staining like recent organisms.

The mode of infection and the portal of entry are unknown; these together with the zoölogical status of the microörganism may yet be ascertained by physicians living in less salubrious regions of tropical America than Panama and in those not yet disturbed by the sanitarian.

EXPLANATION OF PLATES XIX–XXIII.

FIG. 1. Free parasites in film preparation from lung of Case I. Magnified 2,000 diameters. Beside the parasites is a cell containing granules.

FIG. 2. Same magnified 3,000 diameters.

FIG. 3. Mononuclear cells from lungs of Case I, containing many parasites. Magnified 1,000 diameters.

FIG. 4. Pulmonary nodule from Case I. Low power.

FIG. 5. Peripheral sinus in lymph node containing phagocytic mononuclear cell enclosing many parasites. Case I. Magnified 1,000 diameters.

FIG. 6. Section of ileum showing parasites in connective tissue surrounding crypts. Case I. Magnified 1,000 diameters.

FIG. 7. Necrosis of the liver. Case III. Endothelial cells containing large numbers of parasites. Magnified 1,000 diameters.

FIG. 8. Periportal cirrhosis. Case III. Many parasites in large cells within new connective tissue. Magnified 500 diameters.

FIG. 9. Film preparation from the liver. Case III. Endothelial cells containing many parasites. Magnified 1,000 diameters.

FIG. 10. Mononuclear cell in blood space of spleen. Case III. Magnified 1,000 diameters.

FIG. 11. Nodule in intestine. Case III. Endothelial cells containing many parasites. Magnified 1,000 diameters.

I am indebted to the kindness of Dr. Edward Leaming of the Rockefeller Institute for the photographs.

AN EXPERIMENTAL GLOMERULAR LESION CAUSED BY VENOM (CROTALUS ADAMANTEUS).[1]

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PLATE XXIV.

In the course of a recent study of experimental edema (1), rattlesnake venom in small doses was employed, on account of its well-known hemorrhagic action, to bring about the very necessary factor of general vascular injury. For this purpose it proved most satisfactory, without, as a rule, the production of widespread hemorrhages or serious injury to the kidney. The latter, however, in a few instances, presented a very striking and interesting exudative glomerular lesion without evident alteration of the tubular epithelium. It is upon this observation and the experiments to which it led that the present communication is based.

The lesion in question is of double interest: first to those concerned in the experimental study of nephritis; and secondly, to those who have attempted to differentiate the various toxic bodies of snake venom. The recent renewed interest in the study of nephritis by experimental methods centers mainly in an attempt to distinguish between the disturbance due to tubular and to glomerular injury. The important renal poisons affecting only the glomerulus are limited, practically, to cantharidin and arsenic. The addition to this group of venom, which apparently acts through its endotheliotoxic properties is a matter of no small importance to those interested in experimental renal pathology.

On the other hand the lesion throws considerable light on the action of one of the toxic principles of crotalus venom. It may be recalled that in venom poisoning, experimental or otherwise, hemor-

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication April 5, 1909.

rhagic manifestations are very constant. Mitchell (2), in his first investigation published in 1861, offered no explanation of this lesion, but in his second communication in 1868 (3), as the result of a series of experiments in which venom was applied directly to the exposed mesentery and its action studied under the microscope, he came to the conclusion that hemorrhage is due to the direct action of venom on the vessel wall. "When the venom passes through the peritoneum, it so affects the walls of the capillaries as to allow of their rupture and of the consequent escape of blood." Later, in a third investigation, in association with Reichert (4), it was demonstrable that the hemorrhage occurred not from the arteries and veins but from the capillaries only. At this time they could not find actual breaks in the capillary wall and describe the escape of blood as apparently a filtering of the blood through the capillary wall.

No further important contribution to this subject was made until 1902 when Flexner and Noguchi (5) published the results of an extensive study of the constitution of snake venom. In addition to the hematoxic and neurotoxic principles they recognized as the chief toxic constituent of crotalus venom, an endotheliolytic body to which they gave the name "hemorrhagin" and this they considered responsible for the hemorrhages. They found the hemorrhages limited to the capillaries and small veins. Although the extravasation took place through actual rents in the vessel wall, these breaks in continuity were not simple ruptures for they observed microscopically a disappearance as though by solution, of the parts of the wall at the site of hemorrhage. They therefore considered hemorrhagin as a cytolysin for the endothelial cells of blood vessels.

This observation of Flexner and Noguchi has not, as far as I am aware, been confirmed, and when I used venom as a vascular poison to aid in the production of an experimental edema I had no thought of confirming their observation, as I believe I have done, by demonstrating lesions in such an exquisitely delicate endothelial structure as the capillary tuft of the glomerulus.

The glomerular lesion to be described is essentially a vascular lesion, due apparently to a change in the endothelium of the glome-

rular capillaries. It presents two types: the first, extensive hemorrhage into the tuft leading to enlargement and distortion of the tuft with or without rupture; the second, an extensive exudation of the fluid or cellular elements of the blood into the tuft or the capsular space. The cells lining Bowman's capsule show no change while the tubular epithelium generally is either normal or shows only a moderate granular or vacuolar degeneration.

A thorough search of the available literature dealing with venom intoxication fails to reveal an earlier study of these lesions. Several investigators, as first Mitchell, speak of hemorrhages in the kidney visible to the naked eye, or of bloody urine in the bladder, and Mueller (6) describes hematuria in three individuals bitten by the tiger snake, but histologic studies of the kidney appear to be lacking.

The experiments number twenty-one, in all of which the rabbit was used. For the most part the animals received venom alone but a few experiments are included from the earlier work on edema and in these an excess of water was administered by the stomach or chrome salts were given subcutaneously.

The venom used was the dried venom of *Crotalus adamanteus* obtained through the courtesy of Dr. Hideyo Noguchi of the Rockefeller Institute for Medical Research. A standard solution was made with 0.85 per cent. salt solution of such strength that 1 c.c. contained 0.5 mg. of the dried venom. A single dose of 0.5 mg. given intravenously is frequently sufficient to cause the death of a rabbit weighing 1,500 gm. in from five to six days, but in such animals I have not found frank glomerular lesions. These occur more frequently when this dose is given on each of two or three days and almost constantly follow a single dose of 1 mg. or a total administration of 2 mg. in four doses. With a large single dose the animal usually develops hematuria or hemoglobinuria within twenty-four hours, but this rarely occurs with two or three small doses. With the latter dosage, albuminuria of varying grade develops, though generally not until late in the intoxication. As a rule, the animals have been killed when a frank hematuria or albuminuria appeared. This was done, with few exceptions, in order to avoid the rapid post-mortem decomposition, characteristic of venom poisoning, so inimical to proper histological study. As

a result, eleven of the fourteen rabbits with renal injury represent lesions of the fifth or sixth day; the other three represent periods varying from one to four days. The seven rabbits which showed no lesions were animals receiving a single dose or several small doses, and, with the exception of one dying in twenty-four hours and one killed after thirteen days, fall within the three to five day period.

The lesions in the fourteen animals with glomerular involvement may, in a general way, be divided into two groups, one in which exudative phenomena predominate and one in which the characteristic lesion is a hemorrhage into the glomerular tuft. The two lesions may be associated in the same kidney though one or the other type usually predominates in any one kidney. Several factors, as the dose of venom, the period of intoxication, the age (weight) of the animal and the simultaneous administration of other substances as chrome salts or excess of water have all been considered in an effort to determine the cause of difference in type. None of these factors appear to have a determining influence. It it noteworthy, however, that glomerular hemorrhages were very prominent in four rabbits receiving potassium chromate in addition to venom. This salt given alone produces no change in the glomeruli which can be recognized anatomically but does cause widespread injury, with extensive necrosis, of the tubular epithelium. That it may in these experiments have influenced the lesion due to venom is possible but the manner of so acting is not clear.

The material for histological study was hardened in Zenker's fluid and in alcohol, imbedded in celloidin and stained with hematoxylin and eosin, Weigert's fibrin stain and Mallory's connective tissue stain. Frozen sections of formalin-hardened material were stained by Sudan III and hematoxylin.

The hemorrhagic lesion will be described first. Its chief feature is the amount of hemorrhage which may occur in the substance of the tuft without rupture of the outer wall and the escape of blood into the capsular space (Fig. 1). The hemorrhage which may be single or multiple causes a distention of the tuft to two or three times the normal size. The red cells are closely grouped and form, frequently, more or less hyaline masses in which the individual cells

may not be distinguishable. This appearance may be due in part to the agglutination and fusion of cells, as described by those who have studied the effect of venom on the red cells. Sometimes when the hemorrhage is distinctly central the greatly thinned outer wall of the tuft gives the appearance of a thin-walled cyst containing blood. If the hemorrhage is to one side, the remainder of the glomerulus is compressed into a crescent-like mass and the capillaries of the altered tuft contain little if any blood. Many of the larger hemorrhages give, on account of the compression of the rest of the tuft, the appearance of a hemorrhage into the capsular space, but that this is not the case can always be demonstrated by finding a narrow clear space between the cells of Bowman's capsule and the outer border of the tuft. Small accumulations of fibrin or serum may be associated with the hemorrhage but leucocytes are rarely found.

Frequently associated with this definitely intra-glomerular hemorrhage are other glomeruli with free hemorrhage into Bowman's capsule with (Fig. 2) or without the presence of fibrin. In such instances definite evidence of rupture of the tuft is usually seen, the broken fragment of the destroyed tuft sometimes lying free in the midst of red blood cells or more frequently attached to one side of the capsule. At other times the glomerular space is filled with blood without evidence of the destroyed tuft. The coils of the convoluted tubules in the neighborhood of such glomeruli usually contain a considerable amount of blood.

The extent of the hemorrhagic involvement varies. Sometimes nearly every glomerulus is affected; in other animals the ratio is one to two, three, four or five; rarely it is only one to twelve or twenty according to the severity of the intoxication.

The second type of lesion is that characterized by exudation. This may occur alone or may be associated with intraglomerular hemorrhages. All the elements of the blood may appear in the exudate. Red cells frequently form a large part of the picture, but exudates of serum only (Fig. 3) or of serum and fibrin are frequent. Polymorphonuclear leucocytes are seldom seen except as occasional isolated cells (Fig. 4).

The exudate, no matter what its character, distends the capsular

space to two or three times its normal size; the tuft is usually compressed at one side but may remain in its central position. According to the bulk of the exudate either the latter or the tuft may take a crescentic shape. A common picture is that of a delicate fibrin network in which are enmeshed red blood corpuscles and the granules of coagulated serum. Sometimes the fibrin appears as a dense hyaline mass surrounded by red cells (Fig. 1). Some glomeruli contain red cells only or serum only. The kidneys of one animal showed in practically every capsular space a large crescentic finely granular mass of serum. In another, a closely packed fibrinous exudate, homogeneous in appearance and staining deeply with eosin, occurred in three out of every four glomeruli.

Not infrequently the exudate is within and limited to the capillary tuft. Under such circumstances the tuft is transformed into a cavity with a thin wall, giving a ring-like appearance; the cavity of the ring being filled with serum and fibrin (Fig. 5). More rarely, two or three small cyst-like cavities in the tuft contain serum and fibrin threads. At other times the capillaries of the tuft are indistinguishable on account of the presence of strands of hyaline fibrin which give the appearance of a fibrinoid degeneration of the tuft.

It is evident that these phenomena constitute a vascular nephritis and that the primary injury is one affecting the glomerular capillarics and analogous therefore to the vascular lesions occurring elsewhere in the body in venom poisoning and leading to hemorrhage. The glomerular lesion differs from hemorrhage elsewhere in that the phenomena of exudation are added to those of extravasation, or at least can be more readily demonstrated. The question naturally arises: Does the study of this lesion of the capillaries of the glomerulus throw any light on the question of the mode of extravasation of blood in venom poisoning? Reference has been made to Mitchell's observation that the hemorrhage occurs from the capillaries and to the demonstration by Flexner and Noguchi of an endotheliolytic body, "hemorrhagin," in crotalus venom. If such a body exists and if it is responsible for the exudation of the fluid elements of the blood and the extravasation of red cells, the glomerular capillaries should show evidence of its action, and

for such evidence I have searched. Readily demonstrable are the coarser changes, as distention or rupture of the tuft by the accumulation of the blood or exudate, all of which point to weakening of the vessel wall. By careful study may be found finer changes indicative of definite injury to the capillary walls. These changes are seen more readily in connection with the exudative type of lesion. The walls of the capillaries frequently appear swollen and granular with pycnotic or swollen, poorly stained nuclei which are apparently reduced in number (Fig. 2). In some glomeruli the outer portion of the tuft has no distinct sharp outline but an irregular indistinct frayed appearance, as if undergoing solution or gradual disintegration (Fig. 3). Several loops of dilated but empty capillaries sometimes may be seen, usually at the periphery of the tuft, entirely devoid of nuclei (Fig. 1). Others containing blood have a distended congested appearance with a similar scarcity or entire absence of nuclei. Occasionally in the midst of a mass of exudate may be seen a tuft with nuclei in its central portion but none at the periphery. Such a picture is suggestive of tissue erosion or solution. These changes, which do not occur in the ordinary forms of experimental nephritis, suggest, in the absence of evidence of definite tissue necrosis, a disintegration process analogous to autolysis and to be explained by the action of Flexner and Noguchi's endotheliolytic body, "hemorrhagin." In support of this view of the selective action of venom on the glomerular endothelium is the almost complete absence of injury to the tubular epithelium. Casts, in which the products of hemolysis are mingled with exudate, were abundant, but the coarse granular and epithelial casts of tubular origin were not present. In most of the animals the tubular epithelium showed no change; in a few, it was granular and swollen and of the general character of a well-marked cloudy swelling; occasionally, a vacuolar degeneration was evident but at no time necrosis or nuclear destruction and only rarely was fat demonstrable by special stain (Sudan III). This holds also for the cells lining Bowman's capsule, which was almost always well preserved and presented properly staining nuclei. This observation in itself, considered in connection with the extensive lesions in the almost contiguous tuft, would appear to be conclusive as to the selective action of venom.

SUMMARY.

The venom of *Crotalus adamanteus* when administered intravenously to rabbits in properly graded doses causes lesions of the glomerulus of the kidney which may be either hemorrhagic or exudative in character. Both types of lesion are usually associated but either one or the other may predominate. The hemorrhagic lesion, which may be confined to the glomerular tuft, or, by rupture of the latter, may involve the capsular space, is a peculiar localization of the hemorrhage so common in other parts of the body in venom intoxication. On the other hand, the exquisite exudative lesion involving usually the capsular space but sometimes limited, as in the hemorrhagic type, to the tuft itself and with little or no tubular injury, constitutes a type of experimental vascular nephritis, hitherto undescribed, which differs widely in its anatomical appearance from that due to arsenic, cantharidin and other vascular poisons.

As the limitation of the lesion to the glomerulus indicates a selective action of the venom, and as the histological changes in the tuft are suggestive of gradual endothelial destruction and solution, the lesion can be explained by the action of the endotheliolytic body of crotalus venom described by Flexner and Noguchi.

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EXPLANATION OF PLATE XXIV.

FIG. 1. The figure illustrates hemorrhage limited to disintegrated portion of tuft without involvement of the capsular space. It shows also compression of remainder of tuft with dilated capillary loop devoid of endothelial cells. From a rabbit weighing 1,070 gm. which received 0.5 mg. of venom and 0.05 gm. potassium chromate on first and fourth days and was killed on fifth day (albuminuria).

FIG. 2. The figure illustrates hemorrhage into capsular space and presence of large mass of compact fibrin. From a rabbit weighing 1,265 gm. which received 0.5 mg. of venom on first day, 1 mg. on second day and 0.5 mg. on fifth day; killed on sixth day (hematuria).

FIG. 3. The figure illustrates extensive pure serous exudate in capsular space and also the common type of disintegrative lesion of the tuft. From a rabbit weighing 1,260 gm. which received 0.5 mg. venom on the first and third day and 75 c.c. of water by stomach tube daily; killed on fourth day.

FIG. 4. The figure illustrates combined capsular and intra-glomerular lesions with compression of tuft. Intra-glomerular lesion shows maximum accumulation of leucocytes observed in any lesion. From a rabbit weighing 2,010 gm. which received 0.5 mg. on first, second and fifth days and 100 c.c. of water by stomach daily; killed on sixth day.

FIG. 5. The figure illustrates exudative lesion (fibrin and red blood corpuscles) limited to the tuft which, except for compressed portion below, is transformed into a cyst-like body. (From same kidney as Fig. 1.)

All drawings are made from sections stained in hematoxylin and eosin after Zenker hardening and celloidin imbedding ($\frac{1}{8}$ obj., 4 oc., Voightlaender).

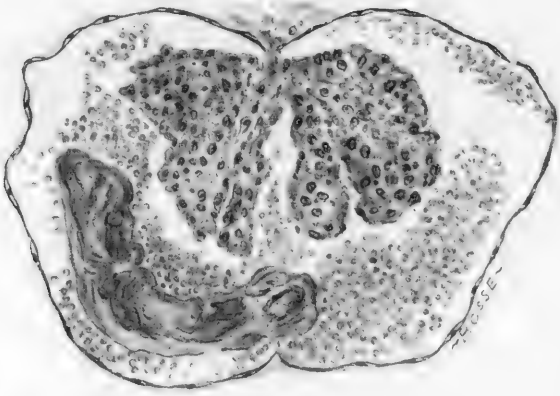
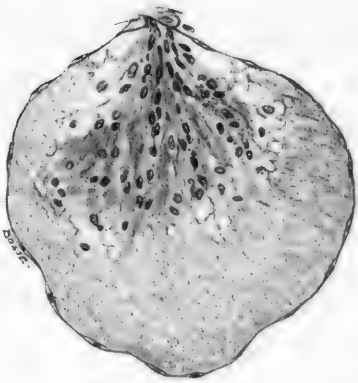


FIG. 2.

FIG. 1.



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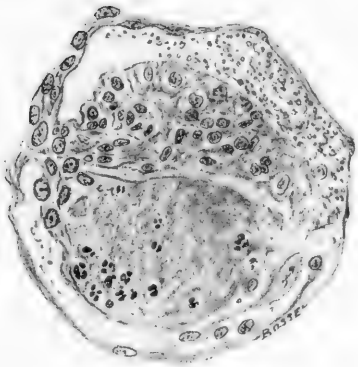

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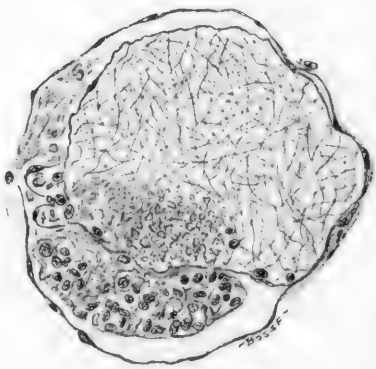

FIG. 5.

BLOOD–PLATELET AND MEGALOKARYOCYTE REACTIONS IN THE RABBIT.[1]

By C. H. BUNTING.

(*From the Pathological Laboratory of the University of Wisconsin.*)

To one who has had the opportunity of examining the preparations of Dr. J. H. Wright, the last word seems to have been said on the subject of the origin of the blood-platelets and the function of the bone-marrow giant cell, the megalokaryocyte. Yet his published conclusions, that the blood-platelets are portions of giant cell protoplasm constricted off from projected pseudopodia, seem as yet not to have received general acceptance and confirmation, and the earlier views as to the nature and origin of platelets still dominate medical literature touching upon the subject. In consequence, the following study of the megalokaryocytes and of the platelets is offered. An investigation of the relation of the megalokaryocyte to changes in other bone-marrow elements was in progress at the time of Wright's[2] publication, but its completion has been unavoidably delayed, and in resuming it, the author has directed his attention especially to platelet relations, as a result of Wright's discovery.

In taking up the problem of the origin of the platelet the first question to be settled is, what is a platelet? If one follows the view of Schwalbe,[3] he is obliged at the outset to admit that platelets can have no single mode of origin, for he says: "For the study of platelets there is no more appropriate material than the human thrombus. There one finds all forms of blood-platelets, those with and those without the inner body, platelets that stain *in toto* with hematoxylin and those that stain entirely with eosin, platelets that contain more

[1] Received for publication March 15 1909. The investigations forming the basis of this report were aided by a grant from the Rockefeller Institute for Medical Research.

[2] *Boston Med. and Surg. Jour.*, 1906, cliv, 643.

[3] *Ergebnisse der allg. Pathol.*, 1907, xi, ii, 919.

or less clearly hemoglobin and those without a trace of hemoglobin." Such a collection of cell fragments is surely heterogeneous; yet, except that it probably includes platelets, such a mass bears no relation to the platelet found in the normal circulating blood. It requires but one carefully and quickly made blood smear from human or animal source, stained by Wright's stain, to show that the blood contains no such varied group of bodies. On the other hand, except for red blood cells and leukocytes it contains but a single element, which always stains the same—the body for which the name platelet has been appropriated. The platelet shows constantly a definite and characteristic picture, a sharply outlined light-blue body mass in which lie a variable number of granules staining metachromatically, a purple in which the red predominates. No platelet stains with eosin, none takes a deep methylene blue nuclear stain, none shows a trace of hemoglobin. The platelets vary much is size, but not in staining reactions. If one checks his stained smear by examination of a fresh blood specimen in sodium metaphosphate, sodium citrate or sodium fluoride mixture, he finds just as many delicate, refractile, oval or lenticular bodies as he has stained platelets (within the limits of experimental error). This is the platelet dealt with in the following experiments and not the cell fragments to be found in every disintegrating thrombus mass, described by Schwalbe.

The experiments here reported have had as their object the determination of the relation between changes in the platelets and changes in the other blood elements in the circulation, and further, to ascertain whether or not the platelet changes followed any law comparable to those governing leukocytic or red cell variations. The rabbit has been the animal used in this work, chiefly because of the ease of doing blood counts on it, and also because of the author's familiarity with blood reactions in that animal. In the experiments careful erythrocyte and leukocyte counts have been made and then the platelet count obtained by the indirect method, *i. e.,* by ascertaining their number relative to that of the red blood cells in fresh preparations and checking this result by the relative number in carefully made stained smears. As a counting medium for platelets I have used ten per cent. metaphosphate of soda solu-

tion, 0.5 per cent. sodium citrate, and 0.2 per cent. sodium fluoride, all of which give parallel results in my experience. The first apparently preserves the platelets the best, if a preparation is obtained free from phosphoric acid. Although the solutions have been checked against each other, to avoid a possible source of error, a single solution has been used throughout a series of counts. In obtaining the preparation for counting, the rabbit's ear has been shaved, cleaned with alcohol, a drop of the counting solution transferred to the ear by a platinum loop, and a small vein pricked through the drop. The mixture of blood and counting solution is transferred by the loop to the slide, where it is mixed with more of the solution previously placed in the slide, is covered, ringed with vaseline and immediately counted under high power. The author has found a high power dry lens combination (Zeiss obj. 4 mm. oc. 12) preferable to the oil immersion series, as the adhesion of the oil to the lens and cover glass it apt to produce motion among the cells of the preparation. In the counts here recorded, the number of platelets has been determined from the number observed while counting approximately five hundred red cells.

The experimental error by this method in the author's hands has been found to be less than 50,000 platelets per cu. mm. This may seem a high error, but it is one which it almost negligible, being but about 6 per cent. of the total count in the normal rabbit and an error which would cause but little variation in the curves of platelet changes produced in the experiments. Throughout the experiments painstaking efforts have been made to obtain accuracy in all counts. By this technique, the platelets of the normal rabbit have been found to number between 600,000 and 800,000 per cu. mm. and the majority of the author's series have given a count nearer the upper figure; that is, above 700,000.

If there exists any parental relation between red blood cell and platelet, it seems to the author that this relation should be shown in the circulating blood, either during regeneration of red cells or following their destruction. To determine this, rabbits were subjected to an initial bleeding and blood counts made at intervals following. The reaction is shown by the following experiment:

RABBIT A-iii (BELGIAN).

April 27, 1908	R.b.c. 5,416,000	w.b.c. 14,750	platelets	730,000
	Bled 30 c.c. from ear vein.			
April 28, 1908	R.b.c. 4,736,000	w.b.c. 12,250	platelets	580,000
April 29, 1908	R.b.c. 4,904,000	w.b.c. 17,250	platelets	940,000
May 1, 1908	R.b.c. 4,760,000	w.b.c. 19,000	platelets	1,080,000
May 5, 1908	R.b.c. 5,368,000	w.b.c. 9,125	platelets	800,000
May 7, 1908	R.b.c. 5,752,000	w.b.c. 13,000	platelets	750,000
	Animal killed.			

A similar reaction is shown by Rabbit A–iv.

RABBIT A-IV (MIXED WHITE AND BROWN).

	Wt. 2,000 gm.			
Jan. 7, 1909	R.b.c. 5,280,000	w.b.c. 8,000	platelets	600,000
Jan. 11, 1909	R.b.c. 5,280,000	w.b.c. 8,000	platelets	600,000
	Animal bled 20 c.c. from ear vein.			
Jan. 12, 1909	R.b.c. 3,880,000	w.b.c. 12,000	platelets	220,000
Jan. 14, 1909	R.b.c. 3,456,000	w.b.c. 8,000	platelets	1,000,000
	Animal killed.			

In these two experiments we have a sharp and definite reaction on the part of the platelets which, upon studying the figures, appears to bear no parallel relation to either the red cells or the white cells. In both experiments there is a fall in the platelets following the hemorrhage; then an increase to a maximum well above the normal, and in the animal allowed to live (A-iii) a return to approximately the normal number. This maximum, a thrombocytosis, if it may be so called, has occurred in both cases before there is any marked sign of regeneration of the red cells; and in the second case, while the red cell count is in fact still diminishing. Nor is any relation to the leukocytes to be made out in either case. The more typical leukocytic reaction is shown by Rabbit A-iv, a slight leukocytosis the day following the hemorrhage with a quick return to normal, the leukocytosis occurring while the platelets are at their lowest point. In Rabbit A-iii there was a preëxisting leukocytosis, the result of an epidemic nasal infection going through the rabbit pen, which somewhat disturbs the count.

As it had been noticed in an animal which had received repeated doses of saponin intravenously that there was a high platelet count (1,400,000 per cubic millimeter), it was decided to use this drug to

produce blood destruction—a toxic anemia. The results were somewhat unexpected, for the saponin destroys not only the erythrocytes, but also the platelets, as shown by the following experiments:

RABBIT S. R. K. (ALBINO).

April 3	R.b.c. 6,696,000	w.b.c. 16,750	platelets	800,000
April 3	Injection of 5 mg. of saponin in ear vein.			
April 4	R.b.c. 3,336,000	w.b.c. 45,000	platelets	120,000
	nucleated r.b.c. 11,000			
April 5	Rabbit died.			

RABBIT S. R. L. (ALBINO).

April 6	R.b.c. 4,968,000	w.b.c. 16,875	platelets	810,000
April 8	Injection of 4 mg. of saponin in ear vein.			
April 9	R.b.c. 2,880,000	w.b.c. 13,780	platelets	140,000
	nucleated r.b.c. 7,300			
April 11	R.b.c. 3,240,000	w.b.c. 17,125	platelets	110,000
April 15	R.b.c. 3,272,000	w.b.c. 11,375	platelets	230,000
April 19	R.b.c. 3,656,000	w.b.c. 7,000	platelets	680,000
April 23	R.b.c. 4,280,000	w.b.c. 8,000	platelets	1,000,000
April 24	Animal killed.			

It is quite apparent from these experiments that destruction of erythrocytes by saponin does not lead to a direct increase in the platelets, or if it does, that they are subsequently destroyed by the drug, and that the high count of platelets originally noted was the result of regeneration of platelets, as evidenced by the gradual increase in Rabbit S. R. L. A point of extreme interest in the regeneration in this rabbit is the greater length of time required for the platelets to reach their maximum number as compared with the time required for them to reach the same point after hemorrhage (Rabbits A-iii and A-iv)—fifteen days in the first, and three and four days in the last two. So great a difference cannot depend merely upon the greater initial reduction in number of platelets. It must depend, it seems to the author, upon a fact pointed out by him in a previous report,[4] and that is that saponin injected intravenously produces an injury to the bone marrow, causing necrosis of cells and tissue and eventually marked scar-tissue formation in the marrow. Such an injury could then influence the regeneration of the platelets only if they were formed in the marrow.

[4] *Jour. of Exper. Med.*, 1906, viii, 625.

Although no relation could be made out in the experiments between leukocytes and platelets, it seemed necessary to put this to further test and several experiments were performed in which sterile inflammations were produced, by the use of croton oil externally, aleuronat in the peritoneal cavity, and turpentine subcutaneously. The experiments showed uniformly an initial fall in the number of platelets and a secondary rise, where the animal was allowed to live. The following rabbit, in which a relatively large dose of turpentine was injected, producing a most extensive subcutaneous exudate, gave the highest reaction in platelets of the author's series, a reaction verified by repetition of the counts in preparations from different veins in both ears and by the stained smears.

RABBIT A-V (MIXED WHITE AND BROWN).

January 19	R.b.c. 6,280,000	w.b.c. 8,500	platelets 720,000
January 19	7 minims of turpentine injected subcutaneously		
January 20		w.b.c. 2,000	platelets 430,000[5]
January 21	R.b.c. 6,944,000	w.b.c. 2,125	platelets 430,000
January 23	R.b.c. 6,352,000	w.b.c. 6,000	platelets 720,000
January 25	R.b.c. 6,000,000	w.b.c. 10,700	platelets 1,700,000
January 26	R.b.c. 6,096,000	w.b.c. 16,000	platelets 1,300,000

Animal killed.

It will be noted here that there is a reduction in platelets during the early stage of the inflammation, while there is a corresponding leukopenia and an increase in red cells, due to the withdrawal of serum into the inflammatory exudate. This is followed by a thrombocytosis which reaches its maximum and has begun to recede, while the leukocyte count is still on the increase. There is then no parallelism between the leukocytes and the platelets.

It would seem from the uniformity of the counts of the blood elements in these experiments, which have been made with the greatest care for accuracy, that the platelets follow a curve independent of the other blood elements; and that in their regeneration they follow the general pathological law of a regeneration in excess, giving a thrombocytosis comparable to a leukocytosis, or to the erythrocytosis which may be obtained in an animal with dry tissues,

[5] Ratio of platelets and red cells established. Number estimated from red count of succeeding day.

as the rabbit. This independence and uniformity of reaction leads naturally to the conclusion of an independent source of origin and the unity of the platelet group.

The experiments, in so far as the counts are concerned, give no clue as to the origin of the platelets, unless it be given by the saponin experiments in which, after the marrow injury, the regeneration of the platelets is delayed. The evidence of the source of the bodies must be obtained from morphological study of the tissues of the animals used, and from the study of blood smears, appropriately stained. This evidence has already been furnished by Wright, and the author's results are in general agreement with his findings.

The tissues of the animals used in the experiments already cited have been carefully studied for evidence as to the origin of the platelets. Fixation for the routine hematoxylin and eosin specimens has been made in Zenker's fluid; for the application of Wright's special stain, in warm saturated corrosive sublimate solution. Attention has been given especially to the bone-marrow and the megalokaryocytes. From counts made for me some time previously, by Dr. R. V. Lamar, it was evident that the megalokaryocytes are increased in number in certain conditions, particularly in inflammations and in regeneration after hemorrhage or toxic blood-injury. To determine this point, in reference to the platelet supply, counts have been made on the marrows of the animals used in these experiments, and of others in which the blood condition was known. It is impossible that these counts should have mathematical accuracy, for two reasons: first, because of the irregular distribution of the megalokaryocytes in the marrow, and second, because of the difficulty of establishing a normal count. Yet they have greater value than the mere impression gained from a casual observation of the marrow, that the cells are increased or diminished in number. For all counts, marrow has been taken from the middle third of the femur, and they are reckoned for an area of one square millimeter of a section 5 μ thick. The normal number of megalokaryocytes appears to be approximately 20 per square millimeter, varying a few either above or below this number in different fields. Rabbit A-v, 7 days after a turpentine injection, and with a platelet count of 1,300,000, showed 53 megalokaryocytes per unit area; Rabbit A-iv,

three days after hemorrhage, with a count of 1,000,000 platelets, showed 47; Rabbit S. R. K., one day after a saponin injection, with platelets 120,000, showed 19; Rabbit S. R. L., 15 days after a saponin injection, with platelets 1,000,000, had 31 per unit area. Counts in animals not included in the earlier part of this report are also of interest. In a rabbit twenty-four hours after an infection with Staphylococcus aureus magalokaryocytes were 12 per unit area: in a second rabbit twenty-four hours after such an injection they were 33; in a rabbit twenty-four hours after an aleuronat peritonitis they were 25; forty-eight hours after a similar injection in another rabbit they were 47, and in another four days after pleural injection they were 37.

When these results are considered, it is apparent that immediately after the establishment of an inflammatory condition or of a toxic anemia in the animal, there is a reduction in the number of megalokaryocytes in the marrow. This reduction is explained by sections from the lung in these cases. It is a fact of common observation that in every case of sharp leukocytic reaction in man a variable and oftentimes large number of megalokaryocyte nuclei are found in the lung capillaries. This is true also of the rabbit. In every case of leukocytosis, and in the reaction to hemorrhage and ricin, and saponin intoxications, a large number of these nuclei are found wedged into the lung capillaries. At times they may get through the lung and may be found in other organs. The demonstration by Askanazy[6] that in the warm stage the megalokaryocyte possesses active amoeboid motion would indicate that this is an active emigration and not a passive washing out or breaking loose of the cells.

The bone marrow deficiency in giant cells thus produced is followed by an increase in their number. Peculiar complex mitotic figures have been found in megalokaryocytes in all of the regenerating marrows studied. Yet I am not ready to answer the question definitely whether these mitotic figures lead to cell division and to an increase in the number of cells, or merely to an increase in the complexity of the nucleus of the cell. At the present stage of my study of the cell, I am inclined to the latter view largely from my failure to find any evidence of either division of the chromatin

[6] Quoted by Schridde, *Anat. Hefte*, 1907, xxiii, 1.

masses or of the cell, while, on the other hand, there are found in these marrows many giant cells with relatively simple nuclei, even merely bi-lobed nuclei. This suggests that these cells develop from a mononuclear cell, and that the complex nucleus of the adult cell is arrived at by repeated but incomplete mitoses. Nevertheless, the occurrence of mitoses in these cells, following the depletion of the marrow, would indicate a heightened activity of these elements.

It may be objected that mere increase in number of megalokaryocytes is not an indication of increased functional activity on their part, and, further, that their increase in number, occurring simultaneously with an increase in the platelets of the circulation, does not necessarily indicate any relation between the two events. This is admitted. Yet in these marrows we find evidence that the giant cells are active in parting with portions of their protoplasm. In fact, we find that a high percentage of them have either no discernible protoplasm or only a narrow rim with ragged outline. For example, in the rabbit A-iv, in the regeneration following hemorrhage, 46 per cent. of the megalokaryocyte nuclei were either without protoplasm, at least as far as could be made out, or had but a slight ragged rim about them. Likewise in Rabbit A-v, in the regeneration after the turpentine inflammation, there were 38 per cent. of such nuclei. This sign of activity is found also in marrows of normal animals, for it is apparently an indication of the normal function of the megalokaryocyte. For the determination of the manner in which the giant cell loses its protoplasm, and the relation to the platelet, specimens of marrow prepared according to the Wright corrosive-acetone-turpentine method and stained by Wright's stain are very satisfactory, although confirmatory evidence is given by Zenker-hematoxylin-eosin specimens. I have not succeeded in obtaining the characteristic staining of the megalokaryocytes in the marrow of the rabbit by the Wright method after methyl alcohol fixation. With corrosive sublimate fixation, however, the staining is usually successful and the picture is definite. The protoplasm of the cell takes a delicate clear blue tint, and in it are imbedded granules with a metachromatic stain. The granules vary in number and distribution. In some cells they are massed about the nucleus, in others they are more or less evenly distributed,

while in still others they tend to be massed toward the periphery and are more or less clumped. The staining reaction is identical with the staining of the protoplasm and granules of the platelets in the circulation when stained in a blood smear with the Wright stain. This identity of staining reaction in the two elements is particularly well brought out in marrow and lung from the same animal carried through the same fixing and staining solutions. In animals with a high peripheral platelet count, the lung capillaries are crowded with platelets, and the observation of them in this situation is rendered easy by the very thin sections one can obtain of the organ. This establishment of the identity in staining reactions of the protoplasm of the megalokaryocytes and its projected pseudopodia and of the platelets is the most valuable feature of the Wright method.

The author has studied with care a series of marrows prepared both by Wright's method and by the Zenker fixation and has found every evidence of the formation and separation of pseudopodia from the megalokaryocyte. These features are most prominent in active marrows, by which is meant marrows taken from animals while the platelet count was increasing or at its maximum, and they are most evident in marrows fixed as soon as possible after the death of the animal. I can understand Schridde's failure to confirm Wright's findings only from the fact that he used chiefly human material obtained at autopsies performed some time after death. In the rabbit these megalokaryocyte projections or pseudopodia vary much in size. One finds short projections of about the diameter of an ordinary platelet, but more often they are from two to three times that width and of considerable length. One can find also every stage of the constricting off process from its inception to its completion with entire separation of the mass. In the highly active marrows, the pseudopodia are often of considerable size, even approaching half of the cell volume. One remarkable picture was seen in which such a large mass was still connected with the protoplasm surrounding the nucleus by a long drawn-out slender neck, while at its distal end it was breaking up into masses of platelet size. Slender pseudopodia of great length were found in the marrow capillaries, and in one animal similar large masses were found in the lung capillaries, one with an area approximately three times

that of a red cell and showing a central constriction as if in process of segmentation. This mass was clearly not the result of fusion of platelets, as was shown by the arrangement of the granules in it, by the sharp outline and by the fact that the platelets in this lung specimen existed as clear cut individuals and not as fused masses. It is apparently by the stripping off of such large protoplasmic masses that there is left in the active marrows so high a percentage of almost naked megalokaryocyte nuclei.

These marrow and lung findings correspond well with what has been noted constantly in the fresh and stained preparations of the circulating blood made from the animals used in these experiments. The observation has been that in the animals with high counts after a rapid rise in platelets, the platelets have shown a great variation in size with a tendency to a large average diameter and with the occurrence of some very large forms. A recently examined blood smear from a case of Hodgkin's disease, in which there was an unusual number (1,140,000) of platelets,[7] showed that this variation in size of platelets and the occurrence of large forms is not confined to animals, but may take place also in human beings. This smear was very successfully made from the platelet standpoint and showed them well preserved, with sharp outlines and not fused into masses. The size of the platelets was very variable. Some were long and narrow, others more oval. Two of the long, narrow platelets were fully 15 μ in length, and one oval form noted especially was of similar length and of a diameter almost equal to that of a red cell. From the sharp cut outlines of these platelets and from the distribution of the granules, there was not the slightest possibility that they were masses of fused platelets.

As a result of this study of the platelets and the megalokaryocytes the author feels justified in drawing the following conclusions:

1. The platelets of the circulation form a group of elements of uniform structure and of common origin.

2. In their regeneration after destruction or loss from the circulation they follow the usual pathological law of regeneration in excess, producing a thrombocytosis comparable to a leucocytosis.

[7] The discussion of platelet relations in Hodgkin's disease is reserved for a subsequent report.

3. This curve of regeneration shows no parallelism to that of the erythrocytes or of the leucocytes.

4. Synchronous with or preceding the appearance of an increased number of platelets in the blood stream, the megalokaryocytes of the bone marrow are increased in number.

5. The megalokaryocytes, by separation of pseudopodia of various sizes, become reduced to almost naked nuclear masses.

6. These pseudopodia are identical in staining reactions with the platelets, and do in fact form them by further segmentation.

7. The megalokaryocyte is the only source of the blood platelets.

EXPERIMENTAL LIPEMIA IN RABBITS.[1]

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PLATE XXV.

INTRODUCTION.

The phenomenon here briefly discussed was observed by the writers during some comparative studies of experimental anemias undertaken for class demonstrations. It was our intention to study with a group of students the changes in the blood of rabbits induced by (*a*) repeated daily hemorrhage and (*b*) poisoning with pyrodin.

In conducting the experiments rabbits were kept in metabolism cages and fed on a mixed diet of bread and cabbage, with water unrestricted. The rabbits which received pyrodin were given graduated doses of the drug, 0.015 to 0.06 gm., by stomach tube, and the blood changes observed by cell counts and hemoglobin determinations daily; smears were made with each count for morphological study.

The rabbits with hemorrhage were bled from the ear veins in amounts varying from 15 c.c. to 45 c.c. each day, averaging about 25 c.c., over long periods. The bleeding was done at the same time each day and in constant relation to feeding. It was observed after bleeding during a number of days that the serum was acquiring an increasing opacity, eventually taking on an appearance resembling rich milk or cream. It was then determined to study this phenomenon in more detail.

APPARENT RELATION OF MILKY SERUM TO HEMORRHAGE AND PHYSICAL CONDITION OF THE RABBITS.

The rabbits under constant conditions of diet, as above stated, were bled from the ear veins six days a week, an average of 25 c.c.

[1] Received for publication April 25, 1909.

per day. And in this way it was noted that, when the count was reduced to about 2,000,000 red cells per cubic millimeter and so maintained, there was a marked loss of body weight and the gradual development of the lipemia to an intense degree. With a day's respite there was constantly a gain of 500,000 to 1,000,000 cells, and increase in weight with a marked diminution in, or disappearance of opacity. Bleeding during eight to sixteen days was required to produce the milky serum, as rabbits showed a considerable individual variation in this respect.

These rabbits killed after prolonged bleeding, showed emaciation with entire disappearance of gross body fat, even about kidneys and heart. Except for a transient weakness immediately following the bleeding, the rabbits appeared well and active. They showed a very marked thirst, increased appetite and active excretion of urine. The feces were normal in appearance. Lastly, when the bleeding was kept up for many weeks the animal became a mere skeleton and the serum gradually lost its milky character. One animal lost 1,468 c.c. of blood in seventy bleedings.

After a short period of rest and gain in weight, the phenomenon was easily reproduced. In one rabbit which was apparently normal after a rest of eight months, during which time it was fed on green vegetables, the serum became intensely milky after a few hemorrhages of 30 to 45 c.c. on successive days.

It was interesting to note that the rabbits which received pyrodin with the same or lower cell counts did not at any time show lipemia In one of our animals, which died during the experiments from excessive bleeding, we tested the tissues for lipase. Loevenhart's ethyl butyrate method was used in the tests. We found lipase present in the liver, spleen and muscles, the amounts being greatest in the order named.

CHARACTERISTICS OF THE MILKY SERUM.

It was constantly observed, as in other anemic blood, that the relative volume of clot was small (see Plate XXV) and from 50 to 65 per cent. of serum was spontaneously separated from the blood on standing. When the opacity was at its height the uncoagulated blood had a definitely whitish tinge as it flowed from the

ear. The serum was quite opaque, and the fresh blood laked with hydrochloric acid for determination of hemoglobin, by Sahli method, showed a cloudy serum, and a consequently higher reading than that of the correspondingly anemic rabbits which received pyrodin.

This milky serum was little affected by strong centrifugation (3,000 revolutions). Only occasionally a thin line of denser "cream" separated in this way or on standing in the ice-box. Microscopically, under a magnification of 1,000 diameters, fine dust-like, slightly refractile droplets could be made out. These did not stain with osmic acid or with Sudân III, even on prolonged contact; thus marking a difference from the fat droplets seen in lipemia after ether narcosis, ingestion of fat, or in diabetes mellitus. A few coarser and more refractile fat droplets, such as are seen in normal serum, stained characteristically with these stains.

On treating the serum with ether it was but slightly altered, even after repeated agitation in a separator funnel for days. The same negative result was obtained with carbon disulphide, carbon tetrachloride and chloroform. In none of these substances was fat extracted to any appreciable extent. With the three last mentioned a permanent emulsion could be obtained by shaking. Here again was a striking contrast to the forms of lipemia previously recorded, as in these the fat is readily removed from the serum by simply washing with ether.

It was not until the close of the experiments, when our material was almost exhausted, that, through a suggestion of Dr. Loevenhart, we found a method for the direct washing of the serum by the previous precipitation of calcium. To effect this change ammonium oxalate crystals were added to the serum in excess and allowed to stand twelve hours, when a fine crystalline precipitate of calcium oxalate was thrown down. Now, after washing with ether, the serum became clear and normal in appearance, and, on evaporating the solvent, a large residue of fat was obtained (see Table III at end of the article).

Before this method was tried, however, Soxhlet extractions had already revealed the fatty nature of the serum and in fact, the chemical study of the fats appended in the table below is based largely on material obtained in this way. For this purpose the

serum was treated with five volumes of absolute alcohol for twelve hours. The protein precipitate was collected in a filter, washed, and dried in a dessicator. The filtrate and washings were united, distilled under reduced pressure, and the fat taken up in pure ether. This ether was put in the receiver of a Soxhlet apparatus and the dry precipitate in the capsule and the extraction continued for from eighteen to twenty-four hours. The ether solution of fat was then evaporated in a weighing dish and the resultant fat weighed and used for further chemical tests.

This ethereal extract consisted of a pale yellow oily substance, of low melting point, and numerous white particles about 1 mm. in diameter which, on microscopic examination, showed the acicular needles and characteristic arrangement of fatty acid crystals. The melting point of the white crystalline masses was definitely higher than that of the non-crystalline oil. On exposure to light and air for several days the fats became darker and slightly rancid.

The fat obtained from different lots of serum by this process varied from 2 to 4.5 per cent. by weight of the serum (see Table III). Only a few direct extractions after decalcification could be made with the material at our disposal and these showed from 2.4 to 2.5 per cent. fat. No attempts were made to crystallize fractionally or separate the individual fats as all the described methods required larger amounts of fat than we had for study. The variation in the percentage of fat obtained is due to the fact that the serum from individual bleedings was used for extraction, and the opacity of the serum varied greatly from day to day.

In order to get some idea of the general character of the fats we determined some of the "constants" used in commercial fat analysis (see Tables I and II).

The *saponification coefficient* is the potassium hydroxide neutralized, expressed as percentage per gram of fat.

Iodine absorption is represented by the actual percentage of iodine absorbed by the fat when digested with a standard alcoholic solution of iodine (Hubl's solution) the excess being titrated with standard thiosulphate solution. This factor is accepted as bearing a constant relation to the unsaturated fatty acids or their glycerides,

and is utilized for the identification of specific fats, as butter, lard, beef fat, etc.

The *acid value* is determined by the number of milligrams of potassium hydroxide required to saturate the free fatty acids in one gram of fat.

In addition, lecithin was determined indirectly as phosphorus pentoxide and calculated as dioleyllecithin, and tests were made for cholesterin and volatile fatty acids. A determination of specific gravity was made. The melting point was roughly estimated for the mixed fats. Parallel extractions were made of normal serum, but no constants determined as the amounts were too small to permit analysis.

In the direct extractions after decalcification, the calcium oxalate with the clear serum, was ashed. The calcium was separated from phosphoric acid, reprecipitated as calcium oxalate, and titrated with standard permanganate.[2] In this way the calcium content of the serum was found to be from 0.165 to 0.176 gm. per 1,000 (normal 0.116),[3] an increase of about 46 per cent.

While certain of the physical and chemical properties of these fats are empirically measured, we have, as yet, no definite knowledge of their actual structure. The iodine absorption indicates a high percentage of unsaturated fatty acids.

CONCLUSION.

In reviewing the literature, no description of a lipemia occurring in relation to simple hemorrhage was found, so that the observation of the phenomenon here recorded would seem to be new.

Very high percentages of fat have been found in the blood of diabetics. Fischer's[4] case showed 18.1 per cent total ether extract. Of this very little was free fat (0.0018 gm. potassium hydroxide per gram of fat); iodine absorption was 60.6 per cent.; cholesterin, 2.6 per cent. Chatin's case, cited by Fischer, showed 1.2 per cent. cholesterin, 66.5 per cent. olein, 32.2 per cent. margarin in the

[2] For method see Boggs, *Johns Hopkins Hospital Bull.*, 1908, xix, 201.
[3] Abderhalden, *Ztschr. f. physiol. Chem.*, 1898, xxv, 65.
[4] Fischer, *Virchows Arch.*, 1903, clxxii, 30, 218.

fat. Neisser and Derlin[5] in the ether extract of blood from a patient with diabetic coma found 19.7 per cent. fat, with melting point of from 39° to 41° C.; iodine absorption was 53.6 per cent. Javal[6] in a similar case found 25.4 per cent. of fat in ether extract of dry serum (perhaps by Soxhlet method); 21 per cent. of the fat was lecithin.

Bleibtreu[7] produced alimentary lipemia in geese by feeding barley and butter. Ether extract of serum showed 6 per cent. of fat. The serum was milky with invisible droplets. Iodine absorption was 57 to 58 per cent. The fat was quite different, chemically, from the fat in the food. Lipemia disappeared a few days after discontinuing the forced feeding.

Our experiments suggest, by analogy, the possible occurrence of lipemia in human anemias. In this connection it is of interest to note that we have recently demonstrated a moderate lipemia in a case of marked secondary anemia from hemorrhoids. The emaciation in such cases, as contrasted with the well-recognized conservation of the fat in pernicious anemia, suggests in human pathology a still further analogy which we now have under investigation.

The fat in our lipemic rabbits differs from fats described above in its insolubility, as well as in its "constants." The change after precipitation of calcium from the serum suggests that the fat may be present in the serum as a protein-calcium-lecithin combination which is decomposed by decalcifying.

While we are not prepared to offer an explanation of the mechanism of this lipemia, it is possible that the great loss of tissue proteins may have some influence on the abnormal fat metabolism. That the fat is derived from the tissues is a fair inference when its occurrence in connection with the loss of weight and the previous disappearance of the body fat are taken into consideration. A more careful study of the lipase in the blood and tissues is desirable. It may be that lowered oxidation following great loss of red cells plays a part.

[5] Neisser and Derlin, *Ztschr. für klin. Med.*, 1904, li, 428.
[6] Javal, *Comp. rend. Soc. de Biol.*, 1908, lxiv, 137.
[7] Bleibtreu, *Deutsch. med. Wchnschr.*, 1907, xxxiii, 446.

TABLE I.

*Constants and other Data Obtained from the Analysis of Fat of Rabbits' Serum.**

	Sp. Gr. at 15.5°C.	Iodine absorption.	Saponification value (KOH)	Free acid.	Volatile acids.	Lecithin.	Cholesterin.
Rabbit I Lipemic	934.5	*a*) 105.6% *b*) 119.8%	*a*) 17% *b*) 22% *c*) 21%	0.84% 0.85%	Traces	10%	None
Rabbit II Lipemic		134.1%	*a*) 19% *b*) 19%		Traces	11.5%	None

TABLE II.

Constants Obtained in the Analysis of Commercial Oils.† For Comparison with Table I.

	Sp. Gr. at 15.5° C.	Iodine absorption.	Saponification value (KOH).	Free acid.
Lard oil	917.0	76.2 per cent.	—	—
Cod liver oil	926.5	166.6 per cent.	18.51 per cent.	0.36 per cent.
Marrow fat (ox)	858.5	45.1 per cent.	19.70 per cent.	—
Linseed oil	934.5	178.8 per cent.	19.28 per cent.	—

TABLE III.

Table of Fat Percentages in Serum.

Rabbit.	Date.	R. b. c.	Hb.	Character of serum	Sohxlet extraction.	Extraction direct from decalcified serum	Calcium content of serum. (In parts per thousand.)
Normal 1	6–V–1908	6,800,000	70%	Clear	0.3%		
Normal 2	7–V	6,200,000	65%	Clear	0.5%		
Pyrodin 7	14–V	2,037,000	33%	Clear	0.49%		
Lipemic I	4–V	2,024,000	29%	Very milky	4.4%		
"	8–V	1,664,000	26%	" "	4.53%		
"	14–V	—	27%	Slightly milky	2.08%		
"	19–V	2,300,000	27%	Milky	3.0%		
"	26–V	1,400,000	20%	Slightly milky	2.3%		
"	4–VI	1,400,000	19%	Clear			
"	9–VI	—	14%	"			
"	‡13–III–1909	—	74%	"			
"	9–IV	—	28%	Milky		2.4%	0.165
"	16–IV	—	25%	"		2.5%	0.176
Lipemic II	16–V–1908	1,700,000	24%	"	2.8%		
"	17–V	1,500,000	23%	"	2.3%		
"	19–V	—	22%	Very milky	3.9%		

* Lipemia has been produced in other rabbits, but analyses of the fats have not been undertaken.

† From Sutton, Volumetric Analysis, London 1904, p. 391.

‡ Experiments resumed on Lipemic Rabbit I, March 13, 1909.

EXPLANATION OF PLATE XXV.

Tubes photographed against black background.

A. Normal rabbit serum. Chloroform at bottom of tube.

B. Fatty serum.

E. Fatty serum washed with ether (*R*) fourteen hours. (1) Slight protein emulsion.

O. Fatty serum treated with ammonium oxalate twelve hours. (*X*) Washed with ether one-half hour. (*L*) Unseparated emulsion of protein. (*Y*) Clear serum with excess ammonium oxalate at bottom of tube. (*R*) Ether containing the fat. (*M*) Fatty serum as separated on standing; (*T*) (*T*) clot showing small bulk of coagulum.

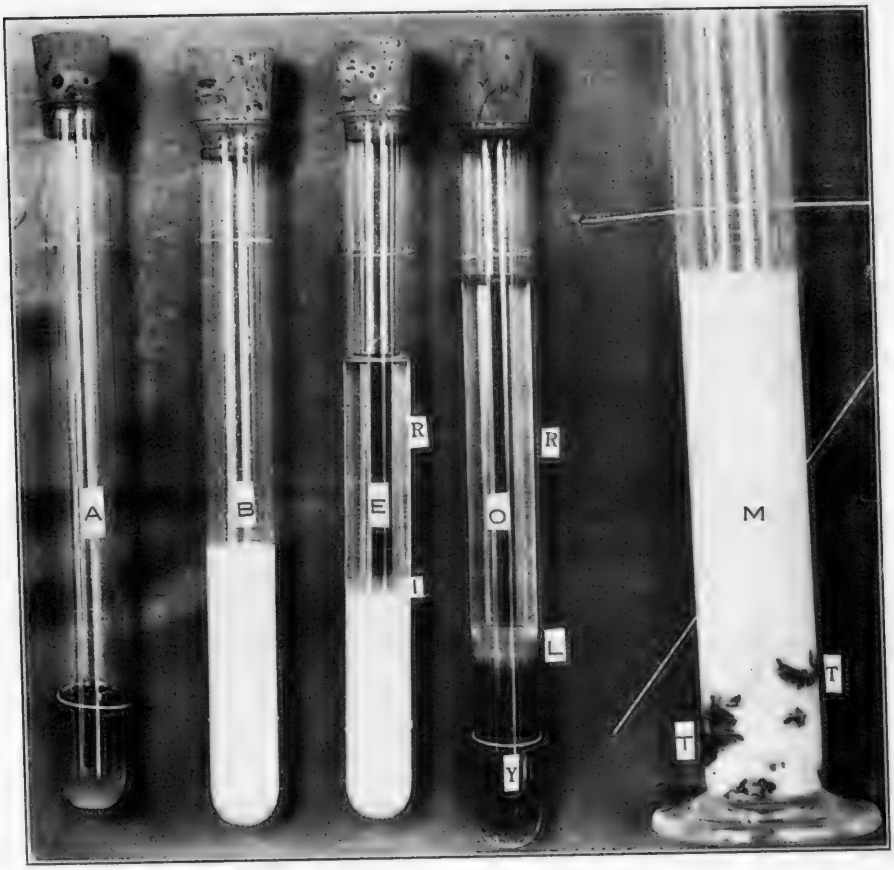

FIG. 1.

DATA CONCERNING THE ETIOLOGY AND PATHOLOGY OF HEMORRHAGIC NECROSIS OF THE PANCREAS (ACUTE HEMORRHAGIC PANCREATITIS).[1]

By EUGENE L. OPIE and J. C. MEAKINS.

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Hemorrhagic pancreatitis was clearly recognized about fifty years ago by Klebs,[2] who believed that the eroding action of the ferments formed by the pancreas was responsible for its occurrence. The classification of acute pancreatitis almost universally adopted, and knowledge of the symptomatology of the various types of inflammation date from the Middleton-Goldsmith lecture of Fitz,[3] delivered before the New York Pathological Society twenty years ago. A certain medico-legal importance has been attached to a disease which may be a cause of sudden unexplained death, but it is perhaps noteworthy that some writers who have emphasized this view have described as hemorrhagic pancreatitis the relatively common post-mortem autolysis which occurs in the pancreas. The cause of the lesion has remained obscure until comparatively recent studies have shown that it can be readily reproduced in lower animals by a variety of means. Human cases have been carefully studied with the aid of these experimental data, with the purpose of defining the cause of the lesion.

It is probable that the physiologist, Claude Bernard,[4] produced the lesion, though he failed to recognize it, years before it had been described in man. It is noteworthy that he injected into the pancreatic duct that fluid, namely, bile, which, as subsequent observations have shown, may cause the lesion in man. In his lectures on experimental physiology, published in 1856, Claude Bernard describes the injection of a mixture of bile and sweet oil into the

[1] Received for publication April 20, 1909.

[2] Handbuch der pathologischen Anatomie, Berlin, 1868, i, 556.

[3] *Med. Rec.*, 1889, xxxv, 197, 225, 253.

[4] Leçons de physiologie expérimentale, Paris, 1856, ii, 278.

pancreatic duct of the dog; the animal died after eighteen hours; there was intense peritonitis and the pancreas was red and contained numerous ecchymoses. Subsequent observers have shown that acute hemorrhagic pancreatitis accompanied by disseminated necrosis of fat can be reproduced by similar injection of a variety of substances. The list of substances which have been successfully employed is long, and suggests the possibility that mechanical injury of injection is sufficient; neverthless, numerous control experiments have demonstrated that various bland substances fail to cause the lesion.

Hlava[5] (1890) first showed that typical acute hemorrhagic pancreatitis with foci of fat necrosis disseminated in the abdominal fat, can be produced in animals by injecting gastric juice through the opened duodenum into the pancreatic duct. Bile (Opie)[6] injected into the duct causes the change; the bile salts (Flexner)[7] are effective, but other constituents of bile fail to produce it. Duodenal contents have been subsequently employed with the same result (Polya).[8] The lesion may be produced with weak solutions of a variety of acids including hydrochloric, nitric and chromic acids, and an alkali, sodium hydroxide, has the same effect (Flexner)[9]; injection of formalin causes the lesion. Sweet oil has the same effect; fatty acids and sodium soaps of these acids have been successfully used, but glycerin is ineffective (Hess).[10] It is difficult to define any common character of these substances, save their power to injure the tissue with which they come into contact. A variety of bland substances have been tested with negative result; these include blood, blood serum (Flexner and Pearce);[11] agar-agar (Flexner), paraffin (Thiroloix,[12] Hess), emulsion of starch (Hess), etc.

[5] *Bull. internat. de l'acad. des sciences de Bohême,* 1898; and *Arch. Bohême,* 1890, iv, 139. Cited by Katz and Winkler, *Arch. f. Verdauungskr.,* 1896, iv, 289.

[6] *Bull. of the Johns Hopkins Hospital,* 1901, xii, 182.

[7] *Jour. of Exper. Med.,* 1906, viii, 167.

[8] *Berl. klin. Wchnschr.,* 1906, xliii, 1562.

[9] Contributions to the Science of Medicine. Dedicated to William H. Welch. *Johns Hopkins Hospital Reports,* 1900, ix, 743.

[10] *München. med. Wchnschr.,* 1903, l, 1905.

[11] *Univ. of Pennsylvania Med. Bull.,* 1901, xiv, 193.

[12] Thesis, Paris, 1892.

Especially significant is the fact that acute hemorrhagic pancreatitis produced by the methods which have been described, may be wholly unaccompanied by bacterial infection. Injection of cultures of various bacteria in small amount causes suppurative or chronic interstitial inflammation (Carnot[13] and others). Introduction of fluid cultures in considerable amount into the pancreatic duct may cause the hemorrhagic lesion which is perhaps referable, according to Hlava, to the acid products which they may contain.

Acute hemorrhagic pancreatitis in man has followed various injuries to the gland; fatal hemorrhagic or gangrenous pancreatitis it is well-known has followed immediately localized injury in the epigastrium, the kick of a horse, crushing of the body between the bumpers of two cars, passage of the wheel of a wagon over the body, etc., and in a few instances hemorrhagic pancreatitis with fat necrosis has occurred about the tract of a gunshot wound of the pancreas. Experimental studies have not fully explained such occurrences. A series of ligatures about the gland occluding both blood vessels and ducts causes a similar lesion, usually fatal within twenty-four hours (Katz and Winkler)[14] (Doberaurer)[15]. Injection of various substances which may act as emboli such as air, oil, paraffin (Bunge),[16] into the arteries of the gland cause necrosis of pancreatic parenchyma and of neighboring fat. Simple experimental crushing of the pancreas does not cause the lesion, but crushing associated with ligation of the blood vessels causes a hemorrhagic lesion accompanied by fat necrosis (Levin).[17]

Before attempting to show how these experiments explain the etiology of acute hemorrhagic pancreatitis in man, we wish to point out certain characters common both to the human and to the experimental lesions. Recognition of these peculiarities is essential to a clear understanding of the lesion, but they have not received the attention they deserve. Hemorrhage into the pancreas, believed to have much analogy to cerebral hemorrhage and caused perhaps by weakness or disease of the blood vessels of the gland, has often

[13] Thesis, Paris, 1898.
[14] *Arch. f. Verdauungskr.*, 1896, iv, 289.
[15] *Beitr. z. klin. Chir.*, 1906, xlviii, 456.
[16] *Arch. f. klin. Chir.*, 1903, lxxi, 726.
[17] *Jour. Med. Research*, 1907, xvi, 419.

been designated pancreatic apoplexy. There has been much discussion concerning the relation of hemorrhage and inflammation. Does inflammation occur as a consequence of hemorrhage, perhaps as the result of infection of hemorrhagic tissue, or is hemorrhage the result of severe inflammatory change? Study of the experimental lesion confirmed by the examination of human cases has rendered this discussion unnecessary, for it has shown that both hemorrhage and inflammation are secondary to necrosis of pancreatic parenchyma. The first effect of bile or other substance which causes the lesion, is death of pancreatic tissue with which the irritant comes in contact. Experiments of Flexner and Pearce have demonstrated the surprising rapidity with which this change occurs; one hour after injection of an irritant into the duct of the gland, hyaline necrosis has occurred and inflammatory products have found their way into the necrotic area. Such facts explain the sudden onset of the disease. One of us[18] has described the necrosis caused by injection of bile into the pancreatic duct of an animal, and has found that it affects not only the pancreatic cells, but the interstitial tissue of the gland together with the blood vessels which it contains. Necrosis of the walls of blood vessels explains the multiple hemorrhages which characterize the lesion. Inflammatory changes, moreover, occur only at the margin of the necrotic tissue. These observations show that the essential feature of the lesion is neither hemorrhage nor inflammation, but consists in death of large masses of pancreatic tissue. They emphasize the fact that acute hemorrhagic pancreatitis is not a bacterial infection like pancreatic abscess, but is primarily necrosis of the pancreas usually caused by contact of some irritant substance with the cells of the gland.

The fact that necrosis in mass is the essential feature of the lesion is well illustrated by Case II (p. 571) which is a typical instance of acute pancreatic necrosis accompanied by disseminated focal fat necrosis. This case is noteworthy because macroscopic hemorrhage is almost wholly wanting although the pancreatic parenchyma has undergone almost complete necrosis; a few black spots represent changed blood but there is no diffuse hemorrhagic infiltration of the necrotic tissue.

[18] *Bull. of the Johns Hopkins Hospital,* 1901, xii, 182.

The lesion usually described as acute hemorrhagic pancreatitis has not the characters of an inflammatory process. Widespread necrosis of pancreatic parenchyma is primary and such inflammatory changes as occur are found only at the margin of the necrotic tissue. A clearer understanding of the nature of the disease would doubtless result if the term hemorrhagic necrosis of the pancreas were used in place of acute hemorrhagic pancreatitis.

Similar lesions do not occur in other organs save, perhaps, in the stomach, where impaired vitality of the mucosa is followed by necrosis and subsequent ulceration, often with hemorrhage brought about by action of the gastric juice upon the injured tissue. The analogy is significant for the pancreas too contains an active proteolytic enzyme which unrestrained is capable of destroying tissue. The peculiar necrosis which affects the pancreatic parenchyma is doubtless referable to the trypsin which is contained in the pancreatic cells. Numerous studies have clearly demonstrated that the necrosis of fat which accompanies hemorrhagic necrosis of the pancreas is caused by a second enzyme, the fat-splitting enzyme of the pancreatic juice.

Recognition of the fact that necrosis of tissue is the essential feature of the acute hemorrhagic lesion, explains the relation of hemorrhagic to gangrenous pancreatitis. In individuals who die within from one to three or four days after onset of the symptoms, the gland is swollen and hemorrhagic, but when death occurs after a longer period there are changes in the hemorrhagic tissue so that it becomes black and gangrenous in appearance. It has been customary to describe as separate diseases hemorrhagic and gangrenous pancreatitis, whereas in both the underlying change is death of pancreatic parenchyma, and the two conditions represent two stages of the same lesion.

We have recently had the opportunity of studying three cases of acute hemorrhagic pancreatitis, which will be briefly cited because each one illustrates some important fact concerning the etiology or pathology of the disease.

Case I.—M. K., female, aged 34 years, was admitted to the Presbyterian Hospital, in the service of Dr. Eliot, on November 8, 1908. She remembered no past illness. One brother died of acute pulmonary tuberculosis.

Present Illness.—In February, 1908, the patient was suddenly attacked with severe pain in the epigastrium. The pain was sharp and intermittent and was accompanied by vomiting which afforded some relief. The pain lasted about twenty-four hours and completely disappeared but returned after several days. There had been slight jaundice during the whole illness, but it was most marked during the three days before admission when the urine was dark colored. These symptoms continued for about three weeks, and disappeared. About October 1, 1908, she was again attacked with severe, sharp epigastric pain accompanied by vomiting. The pain was continuous and was increased only by deep respiration. There was frequent vomiting, but no jaundice; bowels were regular and feces were normal in appearance. About the middle of October the pain gradually diminished and almost disappeared. October 24, 1908, she had a severe chill followed by sweating.

On entrance to the Hospital the patient was well nourished but very pale; there was no jaundice. In the epigastrium to the right of the midline was a fluctuating, slightly tender mass, 8 cm. in diameter; the overlying skin had been reddened by poultices. The edge of the liver could be felt on either side of the midline and the mass seemed to be connected with this organ.

On November 9, 1908, Dr. Eliot opened the abdomen and evacuated a large abscess apparently connected with the liver. It contained a large quantity of thick, brownish pus, with very little odor. Agar-agar inoculated with this pus remained sterile. After operation the patient did not improve; she vomited frequently and was unable to retain any food. The wound discharged freely and at times masses of grayish, cheesy, necrotic material were found on the dressings. The finger could explore a cavity about 12 cm. in diameter with walls covered by soft necrotic material; to the left no wall could be felt. Microscopical examination of the mass discharged from the wound revealed only necrotic debris.

On November 30, 1908, a second operation was performed. A large cavity was found extending to the posterior parietes and for some distance both to the right and to the left of the midline. There was very little pus but the cavity was lined with necrotic material. The patient did not improve after the second operation and died December 1, 1908.

Temperature after admission to the Hospital ranged from 100 to 104° F., pulse from 100 to 160, and respiration from 20 to 60.

Urine contained a trace of albumin and a few casts; there was no bile nor sugar; there was no leucin nor tyrosin and the pancreatic reaction of Cammidge was negative.

The white blood corpuscles on entrance to the Hospital numbered 14,800 per c.cm. but diminished to 3,000 per c.cm. Progressive diminution of polynuclear leucocytes with increase of transitional forms, large mononuclear leucocytes and lymphocytes is exhibited by the counts on page 567.

Autopsy.—An autopsy was performed 3 hours after death. In the upper right hypochrondrium from the wound of operation there is a deep sinus surrounded by firm adhesions.

In the fat of the omentum over the transverse colon there are numerous, small, white areas of fat necrosis. There are no such foci in the dependent part of the omentum. In the median line the coils of small intestines are adherent

	Nov. 8.	Nov. 16.	Nov. 18.	Nov. 27.	Nov. 28.	Nov. 30.
Total leucocytes per c.cm	14,800	10,700	15,700	4,800	2,750	3,000
Poly. leucocytes	78.5%	77 %		29%	26 %	41.5%
Transitional	6	6		16	27.5	15
Large mononuclears	2	2		21	4	16
Lymphocytes	13	14.5		31	42	25.5
Basophilic	0	0		0	0	0.5
Eosinophilic	0	0		1	1	1.5
Stimulation forms	0.5	0.5		2	0	0

to the base of the mesocolon; after these adhesions are broken an abscess cavity, extending across the abdomen above the transverse mesocolon is exposed. At the base of the mesentery, especially where the loops of the small intestines are adherent to the transverse mesocolon are large plaques of fat necrosis. The gastro-colic omentum forms the anterior boundary of the abscess cavity. The hepatic flexure of the colon is firmly adherent to the liver.

From the opening in the abdominal wall the sinus with necrotic edges passed directly backwards to the duodenum. Over the surface of the duodenum the sinus with walls of black, soft tissue extends a short distance to the right; to the left it communicates with a very large cavity lying in front of the pancreas and representing in part the lesser peritoneal cavity. This cavity lies in great part below the pancreas and stomach which at the splenic end of the gland are tightly bound together. It extends to the left as far as the spleen and lateral abdominal wall. An extension from this cavity passes downward into the retroperitoneal tissues to the left of the root of the mesentery, and in front of the kidney. Erosion in this region has extended into the abdominal wall and has reached to within about 1 cm. of the surface above the crest of the ilium.

Behind the body of the pancreas, extending upward through the retroperitoneal tissue is a second sinus which communicates with an immense cavity above the stomach and immediately below the diaphragm extending from the under surface of the liver to the spleen. The diaphragm to the left of the midline in contact with the abscess cavity is penetrated by an opening 1.5 cm. across. This opening communicates with a localized pleural abscess (7 by 8 cm.) about which the lung is firmly bound to the diaphragm; its wall is covered by shaggy fibrin and it contains foul smelling pus-like fluid. The pulmonary tissue in contact with this cavity is firmer than elsewhere.

In front of the head of the pancreas is the abscess cavity described above; the surface of the gland is here covered by soft, black tissue. This black gangrenous tissue covers the body of the pancreas half way to the splenic extremity whereas the remainder of the anterior surface of the body and tail is adherent to the stomach and shows neither necrosis nor erosion. The abscess cavity which has burrowed underneath the gland about its midpart dissects the greater part of the posterior surface of the splenic half from the underlying retroperitoneal tissue. The pancreas throughout is firm and on section the lobulation is less clearly defined than usual. On the surface and less frequently in the substance of the gland occur opaque, yellow spots of fat necrosis 2 or 3 mm. across. On section through the abscess wall into the head of the pancreas a narrow zone of fibrous tissue is found between the necrotic abscess wall and

the pancreatic parenchyma. The duct of the pancreas is slightly dilated and patulous.

On opening the duodenum the papilla of Vater is conspicuous and measures approximately 3 mm. in diameter; the circumference of the orifice is 12 mm. The common bile duct is somewhat dilated and 1 cm. above the orifice, measures 16 mm. in circumference. The duct of Wirsung joins the common bile duct 7 mm. from its duodenal orifice.

The gall bladder is small and its wall is thick. It contains eleven, small gall stones measuring from 6 to 9 mm. in diameter. One stone is partially enclosed by a saccule at the fundus. The cystic duct is dilated.

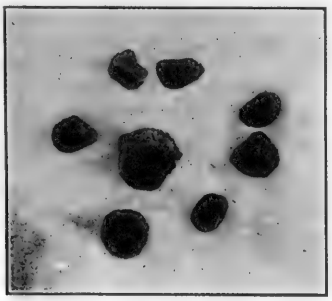

FIG. 1. Showing size of calculi found in gall bladder (Case I). Three stones of approximately same size as the smaller of those shown in the photograph were lost.

The liver weighs 2,275 gm. The surface in contact with the abscess cavity is discolored and covered by necrotic material. The substance is pale and the lobulation is indistinct.

Mucous membrane of the stomach is smooth and pale except for a few submucous hemorrhages. The duodenum is apparently normal. The jejunum is empty.

Culture from the peritoneum contains many varieties of bacteria, including streptococci and B. coli together with other unidentified bacteria. No culture grew from the liver.

Microscopical examination shows that the capsule of the pancreas is much thickened and its outer layers are necrotic. The inner layer consists of vascular granulation tissue in which are hemorrhages. Extending in from the capsule are greatly thickened strands and masses of similar tissue in which there are numerous round cells. In large areas parenchyma is almost wholly replaced and lobules are represented by a few acini. The islands of Langerhans are numerous and large; their capsules are somewhat thickened but there is no increase of connective tissue between the cells. The blood vessels are thickened and hyaline. The walls of the ducts are somewhat thickened; in places the lumen is dilated.

Anatomical Diagnosis.—Hemorrhagic necrosis of the pancreas in process of healing; disseminated fat necrosis; peri-pancreatic abscess with erosion of retroperitoneal tissue; subdiaphragmatic abscess; perforation of the diaphragm; localized diaphragmatic empyema; pneumothorax. Cholelithiasis; chronic cholangitis; fatty degeneration of the liver. Broncho-pneumonia. Fibro-myoma of the uterus.

Several features of the case are especially noteworthy: First, the gall-bladder contains eleven small stones of almost uniform size and the conformation of the common part of the biliary and pancreatic ducts is such that any one of these stones might block the duodenal orifice of the two ducts and divert bile into the pancreatic duct. Such entrance of bile into the pancreas would explain the gangrenous peripancreatitis found at autopsy. A second feature of considerable surgical interest is the tendency of the peripancreatic abscess, at first limited to the lesser peritoneal cavity, to erode the retroperitoneal tissues. The tendency of such abscesses to erode the tissue in front of the left kidney and to point in the lumbar region, is well illustrated by the present case. Incision in this region for counter-drainage has been at times practicable. The abscess cavity in the case just cited had moreover excavated a passage behind the pancreas, and had formed an immense subdiaphragmatic abscess between liver, stomach, diaphragm and spleen. Erosion of the diaphragm had caused complete perforation and had been followed by localized empyema. Such widespread solution of tissue is doubtless referable in part to the tryptic enzyme set free by the injured pancreas.

No growth of bacteria was obtained from the pus found at operation; cultures from the peritoneal cavity at autopsy contained various bacteria among which were Bacillus coli and streptococci. The leucocytes which during the first week after admission to the Hospital numbered approximately 15,000 per cu. mm. rapidly diminished in number and became subnormal just before death.

At autopsy there was no pancreatic necrosis but evidence of destruction of pancratic parenchyma was furnished by bands of dense connective within the gland. When hemorrhagic necrosis produced experimentally in dogs is not fatal parenchyma which undergoes necrosis is absorbed and replaced by newly-formed fibrous

tissue. The same process has doubtless occurred in the case just described. Superficial necrosis of the pancreas with inflammatory changes has been followed by inflammation of the lesser peritoneal cavity with formation of the immense abscess which has been described.

Several years ago one of us[19] showed that a *small* gallstone lodged at the duodenal orifice of the bile papilla might divert bile from the bile duct into the pancreatice duct. A large stone, on the contrary, would fill the common part of the two ducts and obstruct the outflow from both. The gall stone which causes the lesion usually escapes into the intestine and is lost. In the case of hemorrhagic pancreatitis which suggested the occurrence of the mechanism just described, the stone still occluded the common duodenal opening of the two ducts; the pancreatic duct was stained with bile. The earlier literature of the subject contains similar cases and almost identical instances have been recently described by Bunting,[20] and at a recent meeting of the New York Pathological Society, by Crowell. The gall stone which causes the pancreatic lesion usually escapes into the duodenum but in many recorded cases the gall-bladder has contained a large number of uniformly small stones, any one of which might cause the fatal diversion of bile into the pancreatic duct. In the case which we have just reported the gall-bladder contained such small calculi of almost uniform size (Fig. 1).

It has been claimed that gall stones are not more frequently found in association with acute hemorrhagic pancreatitis than in individuals of similar age dying from other diseases. Among eight cases of hemorrhagic necrosis (acute hemorrhagic pancreatitis), which I have had the opportunity of studying, gall stones have been present in five. Among one hundred and five recorded cases collected by Egdahl,[21] cholelithiasis was present in forty-two per cent. This proportion, which may be regarded as an established minimum, is doubtless small, for in many instances data which have been recorded do not exclude the presence of gall-stones. Their presence has not been noted or a single calculus causing the lesion has been

[19] *Loc. cit.*
[20] *Bull. of the Johns Hopkins Hosp.*, 1906, xvii, 265.
[21] *Idem*, 1907, xviii, 130.

lost. According to the statistics collected by Körte,[22] acute hemorrhagic and gangrenous pancreatitis occurs with few exceptions between the ages of twenty and sixty years. According to statistics of Mosher,[23] carefully compiled from German and American sources, the frequency of gall stones between the ages of twenty-one and sixty years is represented by 8.9 per cent. of all autopsies, less than one-fourth of the proportion found with the acute pancreatic lesion.

Whereas it is not improbable that half, or perhaps more than half, of all cases of hemorrhagic necrosis are caused by gall stones, all cases cannot be thus explained. In the following case which occurred in the service of Dr. Eliot at the Presbyterian Hospital, no gall stones were found at autopsy, and the conformation of the pancreatic ducts was such that a gall stone could not have produced the lesion.

Case II. J. S., was admitted to the Presbyterian Hospital in the service of Dr. Eliot, on October 25, 1908. The patient was male, white, aged 55 years, a coach driver by occupation. One sister died with acute tuberculosis.

The patient has used alcohol in excess; in October, 1906, he had acute alcoholic gastritis and suffered with frequent vomiting and severe diarrhœa; there was hematemesis and considerable melena. The liver was palpable 4 cm. below the costal margin and tender; there was no jaundice. After this illness symptoms of chronic gastritis continued and there was frequent morning vomiting and eructations.

On October 22, 1908, there was sudden, severe epigastric pain followed by vomiting. The pain continued to be severe and extended a little outside the epigastric region. Vomiting was almost continuous and the vomitus had a very dark color but no foul odor. There was constipation. The patient had been dyspneic.

On entrance to the Hospital the patient was almost moribund; the face and hands were cyanotic; there was no jaundice. Temperature was 99.6° F., respiration, 40; pulse, 140. The abdomen was symmetrical and distended but not very tense; it moved little with respirations. There was tenderness and rigidity in the right upper quadrant and in less degree in the left upper quadrant. No mass was palpable. On percussion the right upper quadrant, the upper part of the right flank and the whole of the left flank were dull.

No urine was obtainable for examination.

White blood corpuscles October 25, numbered 9,900 per cu. cm. Differential count: polymorphonuclear leucocytes, 88 per cent.; transitionals, 5 per cent.;

[22] Chirurgische Krankheiten des Pankreas. Deutsche Chirurgie, Stuttgart, 1898.

[23] *Bull. of the Johns Hopkins Hospital*, 1901, xii, 253.

large mononuclear leucocytes, 1 per cent.; lymphocytes, 5 per cent. and eosinophiles 0.5 per cent.

Patient died six hours after entrance to the Hospital.

Autopsy.—An autopsy has been performed fifteen hour after death. The mesentery is studded with small grayish areas of fat necrosis. These areas are most numerous on the right side and many large foci are found in the perirenal fat.

The tail and body of the pancreas are soft, and have a deep, dark red color mottled with lighter, grayish areas. Here and there occur minute whitish spots of fat necrosis. Upon the surface of the body occur a few blackish spots extending into the substance of the gland; they represent the only evidence of hemorrhage. The greater part of the head of the gland shows the same changes and one or two black areas occur; the only part of the gland which has the appearance of normal tissue is situated on the posterior and lower part of the head in contact with the duct of Wirsung.

Liver weighs 3,425 gms. The surface is smooth; the cut surface has a uniform yellow color and the lobulation is distinct. The gall bladder, which measures $11 \times 5\frac{1}{2}$ cm., is distended with rather thick, dark green bile, and contains no stones nor sand. The bile ducts are patent.

Stomach is large; its mucous membrane is in places injected and covered by blackish mucous. The duodenum is normal.

The kidneys show a moderate increase of interestitial tissue; there is general arteriosclerosis. At the apices of both lungs are a few encapsulated, partly caseous nodules. The other viscera are normal in appearance.

Cultures from spleen and liver contain Bacillus coli; aerobic and anaerobic cultures from the pancreas contain only Bacillus coli.

Microscopical Examination of the Pancreas.—A section from the *head* near the duct of Santorini shows complete necrosis and disintegration so that the architecture of the gland is completely lost. In a few small spots parenchyma is well preserved, acini being intact and nuclei well stained. Here interstitial tissue is distended as if by edema, red blood corpuscles are fairly numerous and polynuclear leucocytes occur in small number. At the margin of such areas acini still preserve their form but nuclei have disappeared. In some spots of preserved parenchyma there is slight increase of interstitial tissue. About the duct of Wirsung there is in places necrosis with loss of nuclei but except in a few areas the shape of the acini is recognizable, whereas in the greater part of the section tissue is intact and nuclei are stained. A section from the *body* shows almost complete disintegration similar to that about the duct of Santorini. An area of intact tissue shows considerable increase of connective tissue in great part about the lobules; this tissue contains numerous lymphoid cells and red blood corpuscles and a few polynuclear leucocytes. Tissue in the *tail* of the gland is in part necrotic and disintegrated, in part living. The preserved tissue is in places infiltrated with red blood corpuscles. Well preserved islands of Langerhans occur in fair abundance. Small veins contain thrombi.

Anatomical Diagnosis.—Hemorrhagic necrosis of the pancreas; chronic interstitial pancreatitis; fat necrosis; chronic pulmonary tuberculosis; chronic interstitial nephritis; fatty degeneration of the liver; arteriosclerosis.

In Case II death occurred only three days after onset of the symptoms which were such as usually accompany acute hemorrhagic necrosis of the pancreas. Pancreatic parenchyma has undergone almost complete necroses; there are trivial inflammatory changes at the margin of the necrotic tissue and in the neighboring abdominal fat are widely scattered foci of necrosis; the lesion has the characters found with so-called acute hemorrhagic pancreatitis yet hemorrhage is almost wholly wanting. The altered tissue is not visibly infiltrated with blood and minute black spots sparsely scattered upon the surface of the gland represent the only evidence of ecchymosis. Microscopic examination shows that there has been some escape of blood into the interstitial tissue of that parenchyma which is still intact.

The ducts of the pancreas exhibit an anomalous arrangement. The duct of Wirsung, which joins the common bile duct, is of small size and drains only a small part of the head of the gland; this area appears to be the least changed part of the organ. The duct of Santorini, which constantly enters the duodenum nearer the stomach than the duct of Wirsung, and is usually a small accessory duct (see Fig. 2), is in this instance the chief outlet of the gland, and, far larger than the duct of Wirsung, traverses the entire length of the pancreas (see Fig. 3). The orifice of this duct readily admits a probe about two millimeters in diameter. The gall-bladder and bile passages contain no calculi.

Since the duct of Wirsung is relatively small, whereas the main duct of the gland enters the duodenum about 1.5 cm. from the common bile duct, bile could enter only a small part of the pancreas. On the other hand, it is not improbable that an anomalous duct opening by a relatively wide orifice, might have been the portal of entry for material from the duodenum. The power of such material to cause acute hemorrhagic pancreatitis has been repeatedly shown by experiments. Hlava, who showed that acid gastric contents injected into the pancreatic duct causes the lesion, has suggested that antiperistaltic movements of the intestine might drive gastric or duodenal contents into the pancreatic ducts; there is no evidence that the lesion is produced in this way. The discovery of enterokinase in the intestinal juice has suggested that self-diges-

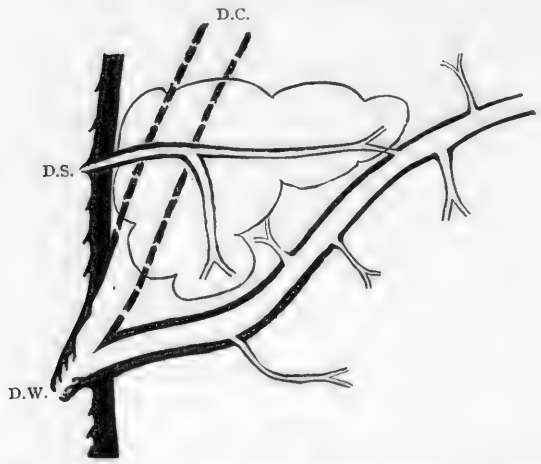

FIG. 2. Diagram showing the usual relative size of the duct of Wirsung (D.W.) and duct of Santorini (D.S.); the duct of Wirsung joins the common bile duct (D.C.).

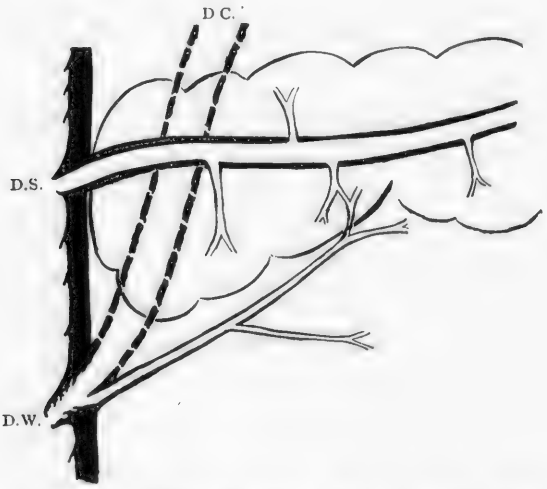

FIG. 3. Diagram showing the relative size of the two pancreatic ducts in Case II. The part of the gland drained by the duct of Santorini is indicated in both diagrams by curved lines.

tion of the pancreas might occur during life should enterokinase find its way into the pancreas. The part played by enterokinase is somewhat doubtful (Pólya, Williams and Busch),[24] but several experimenters (Pólya, Hess) have shown that small quantities of duodenal contents injected into the pancreatic duct will produce acute pancreatic necrosis with fat necrosis.

It is well known that increased pressure within the duodenum does not force duodenal contents into the bile duct or into the pancreatic duct after death. The delicate valves found within the diverticulum of Vater are believed to prevent regurgitation. In approximately one of ten individuals,[25] what is usually the accessory duct of the pancreas, namely, the duct of Santorini, is the chief outlet of the gland and is larger than the duct of Wirsung which joins the common bile duct (Fig. 3). The orifice of such an anomalous duct is perhaps unable to prevent regurgitation of intestinal contents when pressure within the duodenum is increased by vomiting. In the case which I have just described this anomaly has existed and perhaps explains the occurrence of the pancreatic lesion.

Cases recorded in the literature of the subject give some support to this view. Johnstone[26] has described two cases of acute pancreatitis in which the pancreatic duct has opened into the duodenum 1 or 2 cm. from the common bile duct, and although he has described these cases with the purpose of showing that gall stones have not been an etiological factor in the production of the lesion, he does not suggest that the anomalous condition may explain its occurrence. The description of this author makes it probable that the arrangement of the ducts in these two cases resembles that which I have just described. In an additional case described by the same author the distribution of the ducts is not described, whereas in a fourth case the nature of the lesion described is doubtful.

Further evidence that the duct of Santorini may be a portal of entry for irritant material from the duodenum is furnished by a case of Bassett.[27] The two ducts of the gland bore the usual relation to

[24] *Trans. of the Assn. of Am. Phys.*, 1907, xxii, 304.
[25] Opie, Disease of the Pancreas, Philadelphia, 1903, p. 30.
[26] *Colorado Med.*, 1907, iv, 93.
[27] *Trans. of the Chicago Path. Soc.*, 1907, vii, 83.

one another (Fig. 2); the duct of Santorini was much smaller than the duct of Wirsung and drained only a small part of the gland. This duct terminated at the margin of an anomalous duodenal diverticulum of which the mucosa was inflamed. Limited to the immediate neighborhood of this small duct was hemorrhagic pancreatitis with fat necrosis. The patency of the orifice of the duct of Santorini was not demonstrated. In the case which I have described almost the entire gland, of which the chief outlet was the duct of Santorini, was the seat of hemorrhagic inflammation, and the part about the small duct of Wirsung was apparently the least changed part of the organ, whereas in the case of Bassett, with a small duct of Santorini terminating in an anomalous diverticulum, the lesion was limited to the small area drained by this duct. (The domain of the duct of Santorini in the usual and in the anomalous condition is indicated in Figs. 2 and 3.) Although these cases do not afford conclusive proof that duodenal contents causes the lesion, they suggest this possibility with such force that a careful study of the topography of the ducts in cases of hemorrhagic necrosis of the pancreas is desirable.

The etiology in all instances of acute hemorrhagic pancreatitis is not the same. This fact is further emphasized by the occurrence of a small group of cases in which the lesion has so quickly followed abdominal injury in the epigastric region that its relation to traumatism cannot be doubted. A typical case is described by Selberg.[28] A man was kicked over the stomach by a horse and was unconscious for a time. There was pain and gradual distention in the epigastric region together with vomiting. Death occurred after twenty days. The omentum was studded with fat necrosis; the pancreas was infiltrated with blood and was gangrenous in appearance. In the following case localized hemorrhagic necrosis of the pancreas with localized fat necrosis followed a stab wound of the abdomen.

Case III.—J. T., male, aged 20 years, was stabbed on the afternoon of November 4, 1908, in the upper left quadrant of the abdomen with a long stiletto. After about ten minutes he vomited, but there was no blood in the vomitus. Vomiting was followed by nausea. At the site of the wound there was considerable pain, which was increased by breathing.

[28] *Berl. klin. Wchnschr.*, 1901, xxxviii, 923.

On entrance to the Hospital a short time after the injury the temperature was 100.6°; pulse, 96; respirations, 16. The heart and lungs were found normal. Just above the costal margin in the left mammillary line there was a horizontal stab wound one inch long and parallel to the ribs; through the wound projected a mass of omentum, about the size of an egg. There was slight swelling about the wound but very little tenderness. There was no evidence of fluid in the abdomen.

Exploratory operation was performed November 4, 1908. The stab wound in the left costal space perforated the diaphragm, but the pleura was not injured. Upon the antero-lateral surface of the stomach was a perforation 1.5 cm. long through which a small amount of fluid had escaped. The posterior wall was apparently normal. The peritoneal cavity contained a large amount of fluid and clotted blood. The intestines and other viscera were apparently uninjured. The perforation in the anterior wall of the stomach was closed by sutures.

During the succeeding days the temperature varied between 101° and 106°, and the pulse was very rapid. There was frequent nausea and vomiting. There was distention of the abdomen, althought the bowels moved freely and considerable flatus was expelled. Discharge from the wound was profuse. The patient gradually became weaker and died November 9.

Streptococci in pure culture were found in the peritoneal exudate. A blood culture remained sterile.

White blood corpuscles numbered from 3,000 to 7,000 per c.mm.

The urine contained a faint trace of albumin and a few casts.

Autopsy was performed by Dr. Schutz, coroner's physician. Throughout the peritoneal cavity is fibrino-purulent exudate. The wound of the antero-lateral wall of the stomach has remained closed and is in process of healing. There is no perforation of the posterior wall of the stomach, but opposite to the wound in the anterior wall there is upon the mucosa of the posterior wall a circular spot of edema and injection 2.5 cm. in diameter; a minute central area of necrosis about the size of a pin's head, evidently represents the spot in which the point of the knife had struck. Upon the surface of the pancreas immediately behind this wound is a localized hemorrhagic area about 3.5 cm. in diameter and in the fat of this area are conspicuous foci of fat necrosis. Otherwise the pancreas appeared normal.

Microscopic examination of the hemorrhagic area in the pancreas shows hyaline necrosis of the pancreatic parenchyma similar to that frequently observed with so-called acute hemorrhagic pancreatitis; the interstitial tissue is infiltrated with blood and in places contains polynuclear leucocytes. The veins within the hemorrhagic zone are widely dilated and contain thrombi.

It is noteworthy that simple injury to the pancreas of animals fails to produce necrosis, whereas injury associated with occlusion of blood vessels is followed by changes which resemble those of hemorrhagic necrosis (see p. 563). Trivial injury to the pancreas is usually followed by rapid healing but in Case III injury was associated with pancreatic necrosis, hemorrhage, slight inflammation

and focal fat necrosis. It is not improbable that the simultaneous occurrence of localized venous thrombosis and pancreatic injury, both due to a stab wound, explains the occurrence of hemorrhagic necrosis of pancreatic parenchyma.

The lesion usually designated acute hemorrhagic pancreatitis is primarily necrosis of the pancreatic parenchyma and may be caused by various, usually chemical, occasionally mechanical, injuries to the gland. The name hemorrhagic necrosis is preferable to acute hemorrhagic pancreatitis because the lesion is essentially widespread necrosis of the pancreatic parenchyma and the inflammatory changes which occur are secondary to necrosis or subsequent to bacterial infection.

The pancreas is not more susceptible to spontaneous hemorrhage than other organs; so-called pancreatic apoplexy is the result of acute pancreatic necrosis. In some instances pancreatic necrosis (Case II) may cause little if any hemorrhage. So-called gangrenous pancreatitis is a late stage of hemorrhagic necrosis.

Hemorrhagic necrosis of the pancreas is not primarily the result of bacterial infection, but in some instances subsequent infection of gangrenous tissue may cause suppuration.

The most frequent cause of hemorrhagic necrosis of the pancreas in man is penetration of irritant material into the ducts of the pancreas. Bile diverted by a gall stone lodged at the duodenal orifice of the common bile duct has produced the lesion in a large proportion of cases; duodenal contents entering the duct may have the same result. In a small proportion of cases pancreatic necrosis follows injury to the gland and is perhaps in part referable to simultaneous thrombosis of blood vessels.

Certain individuals are rendered susceptible to hemorrhagic necrosis of the pancreas by anatomical peculiarities or anomalies of their pancreatic ducts. In some individuals the passage of a gall stone may divert bile into the pancreas; in others perhaps the structure of the ducts may be such that duodenal contents can find its way into the pancreatic ducts, and thus cause the disease.

A COMPARATIVE STUDY OF THE DIPLOCOCCI OCCURRING IN EPIDEMIC CEREBRO-SPINAL MENINGITIS AND POSTERIOR BASIC MENINGITIS.[1]

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PLATE XXVI.

As long ago as 1898, Still (1) concluded from a study of eight cases of simple, posterior basilar meningitis occurring in infants, that the disease is a sporadic form of epidemic cerebro-spinal meningitis, and that the Gram negative diplococcus found in the meningeal exudate of both forms is identical, such slight differences as greater vitality and lack of virulence being due to natural variation. This view was generally accepted, and the observations made on cases which occurred in this country confirmed Still's findings without adding anything important to our knowledge of the subject. According to the clinical view that posterior basilar meningitis, when not of syphilitic origin, is the chronic stage of an acute cerebro-spinal meningitis (Holt) (2), there seemed no reason to doubt the identity of the causative microörganism in the two affections.

During the past two years, since the recent epidemic of cerebro-spinal meningitis appeared in the British Isles, several observers have claimed that posterior basilar meningitis is a disease due to a specific microörganism definitely and uniformly differing from the meningococcus causing the ordinary epidemic disease, the difference consisting in a total lack of agglutination and opsonin reactions of the diplococcus from cases of posterior basic meningitis with the serum from an epidemic case, and vice versa. Thus in 1907, Houston (3), after studying diplococci isolated from two cases of posterior basilar meningitis at the Great Ormond Street Hospital for Sick Children, which reacted with the blood from these

[1] Received for publication April 1, 1909.

children but not with that from epidemic cases in Belfast, while cocci isolated from the Belfast cases failed to react with the blood from the London cases, concluded that there are two races of meningococci differing in agglutination and opsonic reactions. Houston studied both reactions in the same slide, prepared according to Wright's technique for the study of opsonins, thus using a dilution of one to three. At the onset of meningitis the opsonic index for the meningococcus is low, but it rises gradually. Agglutinins appear about the sixth day, and after that time the two reactions may be studied together.

At the meeting of the British Medical Association held at Sheffield in July, 1908, Houston and Rankin (4) reiterated the view that "Still's disease is due to a meningococcus which has much the same cultural characteristics as the coccus of epidemic cerebro-spinal fever, but differs from it entirely in its opsonic and agglutinating properties." Eve and Clements (5) expressed the same view even more forcibly, stating that the sporadic organism shows little or no tendency to agglutinate or phagocyte in the patient's own blood or in the serum from an epidemic case and will not agglutinate in the Flexner-Jobling antimeningitis serum. Ker (6) was enabled to pick out three cases of post-basic meningitis by means of the agglutination reactions, but one of these cases was very favorably influenced by the Flexner-Jobling anti-serum.

Dr. Flexner having obtained, through the kindness of Dr. Houston, three strains of diplococci from cases of posterior basilar meningitis, kindly gave me the privilege of studying them in comparison with a number of strains from epidemic meningitis cases isolated before and after this study was undertaken. In all, seventeen cultures of diplococci from cases of meningitis were at my disposal. Their histories were as follows:

Two were isolated from typical cases of epidemic meningitis, grown in the laboratory for one and two years.

Two were isolated from typical cases of epidemic meningitis, grown in the laboratory for three months.

Seven were isolated from typical cases of epidemic meningitis, during the course of this work.

One was isolated from the circulating blood of a patient with indefinite meningeal symptoms but without acute meningitis.

Three were obtained from Dr. Houston and regarded as posterior basilar strains.

Two were isolated from typical cases of epidemic meningitis, but were not meningococci.

These cultures were divided into three groups for purposes of study as follows: old, proved strains, recent strains and posterior basilar strains. The two which proved not to be meningococci will be described later.

Morphology.—All were biscuit-shaped diplococci lying in single pairs or in larger groups, and never forming chains. They were not motile.

To Gram's stain they reacted negatively in smears from cerebrospinal fluid, cultures, and in the sections of organs of inoculated animals.

Biology.—On sheep's-serum-agar slants the growth was profuse and typical: greyish-white, smooth, moist showing a slightly metallic sheen on drying, and when moist of a tenacious, mucoid consistency on removal with the needle. On human ascitic fluid agar and on glucose agar, the growth was abundant. On plain agar it was very scant, but readily visible. In plain neutral bouillon a slight turbidity appeared within twenty-four hours and a small, flaky precipitate formed in another day; no pellicle formed. The reaction of the bouillon became alkaline. In serum broth the precipitate was more profuse; the reaction also became alkaline. Litmus milk remained unchanged.

The old, the recent and the posterior basilar strains showed no appreciable differences in any of these culture media. It was not possible to tell one strain or group from another. The two strains designated as not meningococcus were very different. They were also Gram negative cocci occurring in pairs, but they were slightly larger and less strictly biscuit-shaped. They grew profusely on plain agar, caused a marked clouding of serum-glucose agar (the albumin precipitation of Libman) and a diffuse cloudiness of plain and serum bouillon with the formation of a thin pellicle and a thick precipitate. Milk remained fluid. In serum-litmus water containing dextrose, coagulation occurred within twenty-four hours; also in galactose. The other sugars remained unchanged. The facts

that they were Gram negative diplococci and had failed to ferment lactose, mannite, saccharose, raffinose and levulose were their only points in common with true meningococci. The latter showed, in their reactions to sugars in the presence of serum-litmus water, the first points of differences encountered among the various strains; but again they were not race but individual variations. Dextrose, for instance, was fermented by all but the oldest strain, which had lost the power it possessed in this direction eighteen months earlier. The next oldest strain fermented neither dextrose nor maltose, though it had produced acid in both a year before. All the other strains, thirteen in number, fermented maltose. One recent strain, three months old, failed to ferment dextrose at any time. The culture isolated from the blood during life formed acid rapidly in both maltose and dextrose, coagulating the former after three weeks, the latter not at all. The three posterior basilar strains fermented maltose and dextrose, one coagulating maltose only, one producing a sufficient amount of acid in both varieties of sugar to cause coagulation, and the third leaving both sugar media fluid. Arkwright (7) mentions the fact that four strains of meningococcus studied by him fermented no sugars, and Wilson (8) observed the same phenomenon with one strain. The duration of laboratory cultivation of these cultures is not stated. Arkwright gives it as his opinion that the sporadic strains diverge more than the epidemic ones do, as regards the sugar reactions. With this view our observations do not agree. Galactose, lactose, mannite, saccharose, raffinose and levulose were not affected by any one of these fifteen strains of meningococci.

Autolysis was apparent to a marked degree in stained cover slips from all these meningococcus cultures more than twenty hours old, but it was not possible to pick out any one strain as being definitely more or less resistant than another. Suspension of the fifteen cultures in salt solution kept at 4° C. showed no differences in the amount of autolysis present at the end of one, two and three days. The same was true of a series of suspensions kept at 37° C. Nor did the viability of the several strains differ appreciably on solid or in fluid media. One of the older strains invariably outlived all three of the posterior basic strains when kept in the thermostat (37° C.)

on sheep's-serum-agar with or without calcium carbonate. Twenty-four hours after having been heated in a salt solution suspension for thirty minutes at 65° C. the posterior basic cocci were as well preserved as were the older and the recent strains. Evidently the autolytic ferment (9) was the same in all; at least the effects were indistinguishable.

Agglutination.—It is much to be regretted that only one human serum was obtainable for agglutination tests. The early administration of the Flexner-Jobling antimeningitis serum made all other cases under observation valueless for this purpose. The single specimen of human serum came from a seven months' old infant on the second day of an attack of epidemic meningitis, before the injection of the anti-serum. As was to be expected, it gave only negative agglutination reactions with its own strain of meningococci and with the older strains under observation.

The Flexner-Jobling antimeningitis serum remained the only available means for making agglutination tests with these cultures, normal horse serum being used as controls. A recent non-carbolized specimen was obtained. With it, the three posterior basilar strains agglutinated in dilutions of one to twenty only, but five recent epidemic strains did not agglutinate even in so low a dilution. On the other hand, the two oldest strains used for the inoculation of the horses from the first were agglutinated by the serum in dilution as high as one to two hundred, as was the diplococcus isolated from the cerebro-spinal fluid from the case of the infant referred to above, the fluid having been withdrawn within thirty-six hours of the onset of the meningitis and before any serum had been injected. Two facts are brought out by these results: (1) that prolonged artificial cultivation was not the determining factor, and (2) that agglutination reactions with the Flexner-Jobling anti-serum do not serve to differentiate epidemic from posterior basilar strains of meningococci. That all strains of meningococci are not agglutinated in the same dilutions of a serum has been noted by Bruns and Hohn (10). The two non-meningococcus cultures were not agglutinated by this serum in dilutions of one to five, nor was the Staphylococcus aureus.

Phagocytosis.—The final test which the English observers applied to the differentiation of the two races of meningococci was that of

phagocytosis. In this, as in the agglutination tests, I was handicapped by the lack of human serum from patients and convalescents. The only specimen obtainable (vide supra) was taken on the second day of the disease, when its opsonic content was low. The antimeningitis horse serum remained as in the agglutination tests the only available means of study until the inoculation of monkeys was begun. I am aware, therefore, that these tests are not comparable with those upon which Houston based his conclusions. Nevertheless, they are interesting and suggestive. The results with the monkey's serum are given with the protocols of the cases.

Two parallel sets of experiments were made. In one Wright's technique for the study of opsonins, but with diluted serum according to the method of Klien (11), was used; in the other the technique devised by Neufeld and Hüne (12), also with diluted serum, and applied by Neufeld (13) to the standardization of antimeningitis serum was employed. It is based on the observation that immune serum favors phagocytosis because it contains bacteriotropic antibodies which are thermo-stable. The serum was used in dilutions as high as one to five thousand, and the results with both methods were fairly the same. Human leucocytes were used with the Wright technique, those from the peritoneal cavity of a guinea-pig for the Neufeld method. The diplococcal suspensions required were thick. The most striking result of both methods was the variability in the degree of phagocytosis displayed by the same corpuscles to different strains of meningococcus. A table on page 585 gives the results of the first series, made with the Neufeld method.

By the Neufeld method no actual counts are made, but the amount of phagocytosis is estimated and compared in the series of prepared slides, a strain of diplococcus which phagocytes readily, but not spontaneously, being used as a comparison control throughout all the experiments. The opsonic strength of the serum is measured by the dilution at which specific phagocytosis exceeds that which is spontaneous and present in the salt solution control. In Wright's technique two sets of tubes were prepared, one being incubated one and one-half hours, as in the Neufeld method, the other, fifteen minutes only. In the slides prepared from these tubes the engulfed cocci were counted. The results were similar to those given in the

One and One-half Hours Incubation.

	Strain.	Dilutions of anti-meningitis serum.						Salt sol. control.
		1–100	1–200	1–500	1–1000	1–2000	1–5000	
Old	720	+++	+++	++	+	+	±	±
	MSS.	+++	++	+	+	±	–	+
Recent	St. Luke's	+++	+++	+++	++	+	±	±
	Lebanon	+++	+++	++	+	+	±	±
	A. S.	+++	+++	++	+	+	±	±
	Simons	++	+	+	–	–	–	±
	Ward	++	+	+	–	–	–	±
	Fischer	++	+	–	–	–	–	±
	Johnston	++	+	–	–	–	–	±
	Price	++	+	–	–	–	–	±
	T. C.	+	+	–	–	–	–	±
	Silverman	+	+	–	–	–	–	±
Post-Basic	Moseley	++	+	+	–	–	–	±
	Cooper	++	+	–	–	–	–	±
	Windsor	++	+	–	–	–	–	±

above table, showing that one of the recent strains was most susceptible to phagocytosis, two other recent strains and an old strain but slightly less so, while one of the posterior basilar strains was as resistant as two recent cultures, and less so than the other two post-basic strains, which were comparable with five recent strains in susceptibility to phagocytosis in immune serum. Expressed in another way, the opsonic power of this serum was present in higher dilutions for some strains of meningococci than for others, exactly as the agglutinins were present in higher dilutions for some strains than for others. Definite group distinctions are not deducible from these results.

Comparing the results obtained in the specimens incubated fifteen minutes with those incubated one and a half hours, we find that phagocytosis occurs in the same serum dilutions in both, but the number of cocci taken up by the cells increases with the time of exposure allowed them. Thus:

Serum Dilutions.

	Wright's technique, 15′ incubation.							Wright's technique, 90′ incubation.						
Strains.	100	200	500	1,000	2,000	5,000	Salt sol.	100	200	500	1,000	2,000	5,000	Salt sol.
720	4.0	2.5	2.2	1.5	1.0	0.1	0.5	8.0	8.0	3.0	2.5	1.5	1.0	1.0
A.S.	1.9	1.5	1.2	1.0	0.8	0.2	0.2	4.0	3.2	2.5	1.5	1.2	0.3	0.4
Johnston	1.4	0.5	0.1	0.1	0.0	0.0	0.1	2.2	0.6	0.2	0.1	0.1	0.0	0.1
Moseley	2.0	1.4	0.6	0.3	0.2	0.1	0.2	2.9	1.7	1.0	0.4	0.2	0.2	0.5

When normal horse serum was used the control diplococcus ceased to be taken up by the leukocytes in a dilution greater than one in two hundred, while in the immune serum this point was not reached until a dilution of one to two thousand was employed. The phagocytic value of the immune serum for this diplococcus was, therefore, ten times the normal. The results with the other strains in normal horse serum were similar.[2]

The three posterior basilar cultures were inoculated into the spinal canal of monkeys and recovered from them by lumbar puncture. These cultures, after having been passed through an animal, did not agglutinate nor phagocyte to higher degrees than before.

The two non-meningococcus cultures showed no difference in the numbers of organisms taken up by human or guinea-pig leukocytes in the presence of salt solution as compared with antimeningitis horse serum, diluted or in full strength. The phagocytic index of the serum toward the two cultures was practically nil. It has not been possible to identify these two strains of cocci, obtained in pure culture from two different adult cases of apparently typical meningitis. Von Lingelsheim (14) encountered ten varieties of bacteria in his studies with material from patients suspected of having meningitis. These were meningococcus, Diplococcus crassus, staphylococcus, streptococcus, Streptococcus mucosus, Diplococcus mucosus, Diplococcus pharyngis flavus II, Micrococcus cinereus and Gram negative, plump bacilli. From the nine varieties of cocci enumerated, our strains can be differentiated without much difficulty. From Diplococcus intracellularis by its profuse growth on all media, lack of autolysis, fermentation of galactose, and its inability to affect maltose. The cocci under consideration are Gram negative, while Diplococcus crassus is still described by von Lingelsheim (14) as being Gram-doubtful; moreover, the latter ferments levulose, saccharose, lactose and maltose, in addition to dextrose and galactose. The Gram positive staphylococcus, streptococcus and Streptococcus mucosus need no further differentiation, and the morphology and

[2] Dr. Alice Taylor of London very kindly sent serum from two cases of posterior basilar meningitis. Unfortunately the quantity was very small, and the results of an opsonic test (Wright) with one of the posterior basilar strains was entirely negative when the serum reached us. There was not sufficient serum for study with other diplococci.

capsule of Diplococcus mucosus exclude it. Diplococcus pharyngis flavus II, as also I and III form a yellow pigment and ferment levulose instead of galactose. Micrococcus cinereus does not split any sugar, therein resembling Micrococcus catarrhalis, from which its smaller size and less profuse growth serve to differentiate it. That one or more of the diplococci described in the literature as found in air or water may be identical with these cocci found in two specimens of spinal fluid is possible, but the lack of data as to their staining power with Gram's method and their behavior toward the saccharids make it absolutely impossible to give an opinion on the subject. Half an agar culture twenty-four hours old inoculated into the peritoneal cavity of a medium-sized guinea-pig killed the animal in from twenty to thirty hours. At autopsy the point of inoculation was found to be hemorrhagic, the peritoneal cavity contained one or two centimeters of turbid fluid, the omentum was rolled up and hemorrhagic and the liver much congested. The edema about the pancreas, so characteristic of meningococcus infections in these animals, was absent. The pleura and lungs presented nothing abnormal. The cocci were recovered from the peritoneal exudate and from the heart's blood.

EXPERIMENTAL MENINGITIS.

The strains of diplococcus from posterior basilar cases were inoculated into the spinal canal of monkeys, in order to test the pathogenicity of these organisms in relation to meningitis, it having been shown that Diplococcus intracellularis of epidemic cerebro-spinal meningitis causes the disease experimentally in these animals (15).

Experiment I: Strain Windsor.—Two twenty-four hours old sheep-serum-agar slant cultures were suspended in 2 c.c. normal salt solution and inoculated into the lumbar spinal canal of a healthy monkey (*Macacus rhesus*). One hour later the animal seemed ill; in three hours he lay quietly on the floor of his cage, then became restless and evidently very ill. Eighteen hours after inoculation lumbar puncture was performed and a drop of turbid fluid obtained. This showed in films many polymorphonuclear leucocytes, some closely packed with Gram negative diplococci which were swollen and stained poorly. A pure culture of Diplococcus intracellularis was grown from this fluid. The animal was still ill and very weak but improved throughout the next twenty-four hours.

Lumbar puncture on the second day withdrew 1 c.c. of turbid fluid, containing many leucocytes, many intracellular diplococci and more extracellular ones than on the first day. No growth resulted from this fluid. On the following day the fluid was almost clear, and no cocci were found in the films. On the fourth day the fluid was quite clear and the monkey seemed well, though weak. Recovery was complete. The phagocytic index of this animal's serum was examined on five days with the following results. (Wright's method, 1½ hours incubation.)

20 hours after inoculation.			2 days.		3 days.		4 days.		9 days.	
Dilution.	(A. S.)		W.	A. S.	W.	A. S.	W.	A. S.	W.	A. S.
	Strain Windsor.	Recent strain.								
100	6.0	3.4	3.0	2.6	1.6	1.7	1.3	1.0	2.3	2.0
200	4.8	3.2	2.5	2.2	1.0	0.9	0.8	0.6	1.8	—
500	3.2	3.1	—	2.0	—	—	—	—	1.0	0.9
1,000	2.8	3.0	2.4	1.5	1.0	—	—	—	0.8	0.6
2,000	2.5	1.5	1.8	1.2	0.8	1.5	—	—	—	—
Control	0.8	0.7	0.8	0.7	0.6	0.4	0.7	0.5	0.6	0.5

It becomes apparent from these results that a recent (epidemic) strain of Diplococcus intracellularis was phagocyted in as high dilutions of this animal's serum as was the strain of posterior basilar meningitis used for inoculation. The comparatively high phagocytic index one day after the injection, the short duration of the rise and the rapid fall after the second day are also of interest.

Experiment II: Strain Cooper.—(*a*) Three sheep-serum-agar slants, twenty-four hours old, were suspended in 1.5 c.c. salt solution and injected into lumbar region of spinal canal of a monkey. Within one hour animal seemed ill. Died in seven hours. Autopsy—No purulent exudate visible. Vessels of the pia mater intensely engorged over entire brain and cord. Ventricles not dilated. Brain substance of normal color and consistence. Pia-arachnoid over entire cord and medulla, looks turbid. *Films: Cerebral cortex.*—No leucocytes and no diplococci found. *Medulla*—Many leucocytes, in many of which cocci are contained; cocci also outside the cells; autolysis of cocci marked. *Lumbar and dorsal cord*—Enormous numbers of diplococci, both extra- and intracellular; very large numbers of lucocytes. *Choroid plexus*—Few diplococci within and without leucocytes. *Cultures*—No growth from heart's blood or spleen. Pure growths from medulla, lateral ventricles, and spinal cord. *Microscopic examination of sections*—The pia arachnoid of the spinal cord contains an exudate, in some places quite heavy, consisting of pus cells and fibrin. The exudate covers and surrounds the nerve roots and the intervertebral ganglia. The latter seem not to have been invaded. In a few places, probably corresponding with the lumbar puncture wounds, the dura is infiltrated with pus cells. The number of diplococci in the exudate is very large and they are contained chiefly in the leucocytes. The cerebral meninges also contain leucocytes, fibrin, serum in excess

and extravascular red corpuscles. The blood vessels are widely distended. In the depth of the sulci the cellular exudate is replaced by an inflammatory edema with fibrin formation. Over the cerebellum the leucocytic exudate is heavy; within the cortex the perivascular lymph sheaths contain an excess of polynuclear leucocytes.

(*b*) Two sheep-serum-agar slants suspended in 1 c.c. of salt solution were inoculated into the lumbar region of the spinal canal. The animal became very sick within two hours and died in nineteen hours. *Autopsy*—In the cervical region of the spinal cord the pia-arachnoid was edematous and hemorrhagic. Over the rest of the brain and cord there was marked congestion, but no visible exudate. *Films and cultures* as in *Experiment* (*a*). *Microscopic examination of sections*—The exudate in the spinal meninges is less in quantity than in the preceding monkey and it is almost or entirely leucocytic in character. The exudate in the cerebral meninges is also less and consists of a mixture of polynuclear leucocytes and of red corpuscles. The number of diplococci in the leucocytes in the spinal exudate is large; in the cerebral, small.

(*c*) One solid culture, twenty-four hours old, was suspended in 1 c.c. of salt solution and injected into the lumbar region of the spinal cord of a monkey. The animal was weak and ill during the night. Lumbar puncture on the following morning withdrew very turbid fluid, showing many leucocytes crowded with diplococci; also some extracellular organisms. On the second morning 0.5 c.c. of turbid fluid was withdrawn, showing numerous leucocytes and large numbers of well staining diplococci. Cultures grew well and were pure. On the third day there was some improvement in the animal's condition. The fluid withdrawn by lumbar puncture was still turbid, containing leucocytes but very few diplococci. The following day no diplococci were found and on the fifth day the spinal fluid was clear. The monkey was then well. Examination of the blood showed that while before inoculation the meningococcus (strain Cooper) was not taken up by the leucocytes in dilutions exceeding 1 to 200, that on the first and second days after injection they were phagocyted in dilutions of 1 to 2,000, on the fourth day to 1 to 1,000, and on the seventh day not over 1 to 200. A recent strain (A.S.) again parallel with the one used for inoculation. In the slides prepared from the serum on the seventh day agglutination of the diplococci was apparent. In the earlier specimens it had been absent.

(*d*) Two-thirds of one serum-agar-culture was injected into the lumbar region of the spinal canal of a healthy monkey. No symptoms of illness developed, but on the day following the inoculation the spinal fluid was slightly turbid, containing leucocytes with intracellular diplococci in small numbers, and still fewer outside the cells. The cocci grew in pure cultures. The phagocytic index of the serum before the beginning of the experiment was nil in dilutions over 1 to 100. It was apparent in dilutions of 1 to 2,000 after twenty-two hours and had fallen to what it was before inoculation on the fifth day, the recent strain (A.S.) running a parallel curve with the one used for inoculation.

Experiment III: Strain Moseley.—One and a half sheep-serum-agar cultures, twenty-four hours old, were injected into the lumbar region of the spinal canal of a monkey. He became ill within two hours, and on the following

morning was very sick, lying on the floor in his cage. Two cubic centimeters of turbid fluid were withdrawn by lumbar puncture and in it many leucocytes containing well staining diplococci were found; a small number of autolyzed diplococci were also present. Cultures from this fluid grew well. On the following morning opisthotonos was marked; fibrillary twitchings and general convulsions were produced by disturbance. The pupils were irregularly contracted and reacted slowly. The animal was perfectly rigid unless disturbed by a touch or a loud noise, when a general convulsion came on. The cerebro-spinal fluid was very turbid, and contained many well-staining diplococci inside leucocytes and outside them as well. On the third morning the monkey was very quiet, lying on his side. No convulsions and no twitchings occurred. The eyes were less irregular. The fluid was still turbid, with many diplococci in the leucocytes. The condition changed but slightly during the following day; the animal made attempts to sit up but soon resumed the recumbent position. On the morning of the fifth day it died. The symptoms of the first days were very much like those of a human patient with meningitis and the clinical picture on the second day was most suggestive. The photograph illustrates the condition at this time.

At the autopsy, encephalitis of the right frontal lobe was found. This portion of the cerebrum was very bright red in color, raised above the level of the adjoining lobes, translucent in appearance, studded with punctate hemorrhages. The layer of gray matter was double the thickness of that in the opposite hemisphere. The remainder of the brain and cord showed a moderate congestion of the vessels of the pia mater. *Cultures* from the cerebrum, cerebellum and spinal cord showed no growth. The microscopic examination of the sections of the spinal cord and brain of this animal indicates that it had recovered almost completely from the meningeal infection and that it succumbed to the cerebral lesion. There are present small remains of the exudate in the meninges consisting of leucocytes and proliferated mononuclear cells.

The chief interest centers in the encephalitis which proved to be an infarction affecting a large part of the hemisphere. The tissue in the infarcted area is necrotic, the contained blood vessels are necrotic, and nuclear staining is generally absent. Around some of the blood vessels, now thrombosed, are collections of leucocytes also undergoing necrosis, and in various parts are punctiform hemorrhages and beginning calcium salt deposits. The membranes over the encephalitic focus are inflamed; they show an accumulation of leucocytes and pus cells. The larger branches of the veins are closed wholly or partially by thrombi containing fibrin and trans-

formed, fused, red corpuscles. In places a heavier exudate lies between the membranes and the encephalitic tissue of which the larger part is composed of proliferated pial cells of large size, which have taken up extravasated red corpuscles that are undergoing decolorization.

This case is of unusual interest because of its typical clinical picture, its prolonged course compared with the cases described by Dr. Flexner in previous experiments, and the exceedingly severe and unusual lesion found at autopsy. It should be added that hemorrhagic encephalitis as a complication of cerebro-spinal meningitis in human beings is of occasional occurrence (16).

CONCLUSIONS.

The study carried out and recorded in this paper did not lead to the finding of any reliable criteria of difference between strains of Diplococcus intracellularis obtained from typical cases of epidemic meningitis and several cultures obtained from cases of posterior basic meningitis.

The successful experiments made with monkeys show that the diplococcus obtained from cases of posterior basic meningitis is capable of setting up rapidly and acutely fatal forms of meningitis and in producing organic lesions of the cerebral tissues of great severity.

This study would, therefore, suggest that the antimeningitis serum should be as useful in cases of posterior basic meningitis so-called, as it has been in epidemic meningitis, especially if it were employed early in the disease.

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EXPLANATION OF PLATE XXVI.

The photograph which was kindly made by Dr. Leaming shows the monkey of Experiment III in the condition of opisthotonos. The hair was cut away from the back of the neck in order to show the degree of curvature and retraction of the head.

FIG. 1.

THE INFLUENCE OF TEMPERATURE ON HEMOLYSIS IN HYPOTONIC SOLUTIONS.[1]

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During the year 1905–1906 it was my privilege to assist Dr. Theobald Smith in determinations of the resistance of the erythrocytes of horses to hemolysis by water. The method in use was a modification of that originated by Hamburger in which the resistance of the cells is measured by the amount of neutral salt required to protect them against the laking effect of distilled water. Struck by the accuracy of the results obtained and familiar in some degree with the conditions under which the hemolytic effect of the complex hemolysins is determined, I was led to try the influence of temperature on the destruction of erythrocytes in dilute solutions, a phenomenon supposed to depend on purely physical conditions.

The experiments made have shown conclusively that hemolysis becomes progressively more marked as the temperature is decreased from 37° C. to 5° C. Aside from any importance which may be ascribed to this fact, the experiments deserve to be recorded in some detail because they illustrate the superiority in point of delicacy and accuracy of the technique employed as contrasted with the original method on which it is based.

Hamburger[2] is the only experimenter who until very recently had attempted to determine the influence of temperature on hemolysis in hypotonic salt solution. He tested beef corpuscles in solutions of KNO_3, NaCl, and cane sugar in varying concentrations, and at temperatures 0° C., 14° C. and 34° C. He found no difference in the amount of laking at these temperatures. He estimated the

[1] Received for publication April 15, 1909. Presented in abstract before the Society for Experimental Biology and Medicine, New York, February 17, 1909.

[2] The original article (*Arch. f. Anat. u. Physiol., Physiol. Abt.*, 1886, 476) is transcribed without essential changes in this author's Osmotischer Druck u. Ionenlehre, Wiesbaden, 1902, i, 172.

degree of hemolysis by comparison of the point of beginning hemolysis in the separate series. It will be developed later that it was this use of the point of beginning hemolysis which accounts for his failure to detect differences dependent on temperature.

Kiss[3] in the present year publishes observations in accord with those here presented. He used beef erythrocytes only, and his method was such that he was forced to base his judgment on relatively small percentages of corpuscles hemolyzed. This author corrects this mistake of Hamburger's as to the influence of temperature on hemolysis, and undertakes on the basis of his results in this respect and others even more important to combat the whole idea that the erythrocyte is essentially a semipermeable membrane enclosing fluid contents, and that physical and chemical hemolytic agents act in accordance with what would be expected on such an analogy. As will be seen, the present paper was constructed on the older conception as a foundation. As I am not well prepared by experience to discuss most phases of the work of Kiss and as I have been chiefly concerned with method and fact rather than theory, it seems best to allow my paper to stand as written, omitting an extended discussion of the important monograph of this author.

As already stated I have used in my work the modification of Hamburger's method introduced by Theobald Smith.[4, 5] For a detailed description, the interested reader is referred to the original papers. Briefly stated the method is as follows: sodium chloride solutions of graded strength from about .40 per cent. to about .70 per cent. varying by a constant difference of .02 per cent. are carefully prepared and preserved. At the time of use a given quantity of each solution is measured into a small test tube. The tubes used are selected to approach closely a uniform diameter. Each conveniently takes 3 c.c. of fluid. The concentrated red blood corpuscles are added to the fluid from a pipette, the measure being by drop.

[3] Kiss, J., Das periodische System der Elemente und die Giftwirkung, Wien u. Leipzig, 1909.

[4] Smith, T., The Pathological Effects of Periodic Losses of Blood, *Jour. of Med. Research,* 1904, xii, 385.

[5] Smith, T. and Brown, H. R., The Resistance of the Red Blood Corpuscles of the Horse to Salt Solutions of Different Tonicities, before and after Repeated Withdrawals of Blood, *Jour. of Med. Research,* 1906, xv, 425.

The tubes are thoroughly shaken at once. After a given time, preferably several hours, the amount of hemolysis in each tube is ascertained by comparing the color of the supernatant fluid with that in a series of tubes of the same diameter containing varying percentages of hemoglobin. The hemoglobin solution is freshly prepared each time by laking a quantity of erythrocytes equal to that used in each tube of the test series in a quantity of distilled water also equal to that of the salt solution used in each tube. From a multiple of these amounts used as a 100 per cent. hemoglobin solution, suitable dilutions are made. Where experiments are conducted with a single suspension of blood corpuscles no great precaution need be taken to secure comparative results. If the corpuscles of different individuals are to be used, then one must refer each test series to a hemoglobin solution obtained with the particular blood suspension used in that series, or at least assure one's self that the suspensions are of equal value. If normal horse blood is used it suffices to allow the corpuscles to sediment over night in order to attain suspensions of equal hemoglobin content. If the blood of cattle, rabbits or guinea-pigs is used one must centrifugate at high speed to a constant corpuscle volume to attain this result. If the corpuscle suspension is well concentrated the factor of error due to the adherence of a small amount of blood serum is a constant and is too small to embarrass the result.

The experiments here reported were carried out by the following extension of the method as above outlined. A series of tubes was prepared for each temperature to be considered, and filled with 3 c.c. of the varying strengths of salt solution. The tubes of each series were placed in the room, ice chamber, or thermostat as the case might be and allowed to remain half an hour to attain the required temperature. The erythrocytes which had been preserved in the ice chest were in the meantime allowed to attain room temperature. The several series of tubes were brought out separately, the corpuscles added, shaken and returned at once to ice chest or thermostat. The period of temperature inaccuracy was thus but two or three minutes. After five hours, a convenient but not essential period, the tubes were brought out and the percentage of hemolysis ascertained.

The results obtained are shown in the following tables and charts. The few experiments given are but examples of a considerable number which have given the same general result without a single exception.

TABLE I.

Sodium Chloride Solution "C." Erythrocytes of Horse 95, Concentrated, 2 Drops. Time of Exposure 5 Hours.

Per cent. s. s.	Per cent. hemolysis.		
	38° C.	28° C.	5° C.
.40	100	100	100
.42	99	99	100
.44	90	90	100
.46	75	85	99
.48	25	50	90
.50	8	17	70
.52	3	5	55
.54	1.25	2	22
.56	.5	1	7
.58	0	.5	2
.60	0	0	.25
.62	0	0	0
.64	0	0	0

TABLE II.

Horse Erythrocytes. Sodium Chloride Solution.

Per cent. s. s.	Per cent. hemolysis.			
	42° C.	35° C.	20° C.	6° C.
.54	38	8	10	30
.56	38	5	6	22
.85	2	0	0	0

Erythrocytes—Horse 96—2 drops.

Salt solution 3 c.c. After five hours at the temperature given the tubes were shaken and put in the ice box over night. Readings at 20 hours.

That as the temperature is increased from 5° C. to 37° C. the amount of hemolysis is decreased is clearly shown in each instance. This is irrespective of the kind of blood used. It occurs when either sodium chloride or cane sugar is used to give tonicity to the solution. It may also be stated that the difference is clearly marked in the first few minutes of contact of corpuscle and solution, being discernible as soon as the laking has gone far enough to permit

TABLE III.

Calf Blood Corpuscles.

Per cent. s. s.	Per cent. hemolysis.			
	42° C.	38° C.	28° C.	5° C.
.40	—	—	100	100
.42	—	—	100	100
.44	—	—	100	100
.46	100	—	99	100
.48	99	—	98	100
.50	90	90	95	99.5
.52	62	80	82	98
.54	45	60	65	95
.56	25	45	48	85
.58	17	25	32	65
.60	10	12	18	38
.62	5	7	7	20
.64	3	3	4	12
.66	2	—	1	8

Calf blood centrifugalized ½ hr. to constant corpuscle volume, 2 drops. Salt solution "D"—3 c.c. Time 5 hours.

TABLE IV.

Guinea-Pig Erythrocytes. Sodium Chloride Solution.

Per cent. s. s.	Per cent. hemolysis.			
	42° C.	35° C.	20° C.	6° C.
.46	18	20	25	35
.48	8	11	15	23

Guinea-pig cells, 2 days old, centrifugalized to constant volume, 2 drops. Time 5 hours.

Salt solution 3 c.c. Control corpuscles in .85 per cent. s. s. heated to 42° C. Shown hemolysis + 2 per cent.

TABLE V.

Rabbit Erythrocytes. Sodium Chloride Solution.

Per cent. s. s.	Per cent. hemolysis.			
	43° C.	37° C.	26° C.	5° C.
.48	60	85	99	100
.50	45	60	95	98
.52	18	25	35	65
.56	4	5	10	15

TABLE VI.

Horse Erythrocytes. Cane Sugar Solution.

Per cent. sugar.	Per cent. hemolysis.		
	38° C.	27° C.	5° C.
3	100	100	100
3.25	97	98	100
3.50	80	90	95
3.75	65	80	85
4.00	45	55	65
4.25	25	28	45
4.50	15	18	32

Corpuscles—Horse 95—2 drops. Sugar solution 3 c.c. Exposure 5 hours. Ice box. Readings 24 hours.

TABLE VII.

Rabbit Erythrocytes. Cane Sugar Solution.

Per cent. sugar.	Per cent hemolysis.			
	43° C.	37° C.	26° C.	5° C.
4.50	28	25	35	75
5.00	8[a]	7[a]	7[a]	35

Erythrocytes. 2 drops of concentrated suspension.
Sugar solution 3 c.c.
a = pronounced agglutination.

comparison. Furthermore the difference once developed by exposure to the different temperatures is maintained if the tubes are then brought to an equal temperature. Thus, if, after the five-hour reading, the tubes are all shaken and put in the ice box and the readings taken again at the end of twenty-four hours, it will be found that there has been a slight and equal increase in the hemolysis in all tubes but that the difference developed by the first exposure to varying temperatures has been maintained.

The facts shown by the tables may be somewhat further analyzed with advantage. It was pointed out in the work of Smith and Brown above cited that if the hemolysis resulting in different percentages of salt solution be graphically illustrated by plotting the salt solution percentages on the abscissæ, and the percentages of corpuscles hemolysed in each per cent. salt solution as ordinates, the resulting curve in normal horses approaches the form of a simple, unimodal, nearly symmetrical curve with range limited in

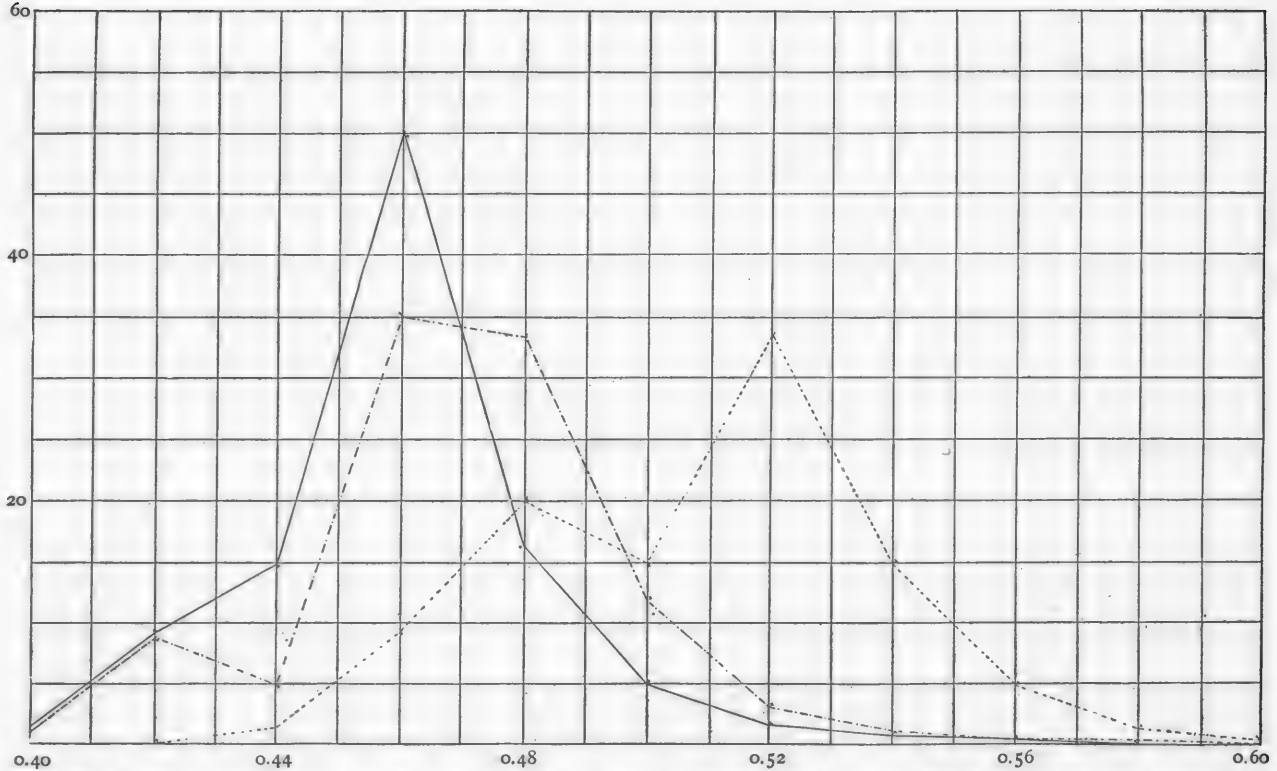

CHART I. The chart has been prepared from Table I; solid line represents 37° C.; broken line, 28° C.; and dotted line, 5° C. Percentages of sodium chloride solution are plotted on the abscissa. Percentages of erythrocytes destroyed between consecutive strengths of salt solution are plotted on the ordinate.

both directions. Chart I is the experiment of Table I represented in this way. It will be noted that Curve *a* representing the laking at 37° C. approaches nearly to the form of curve described as normal for the horse erythrocytes. Moreover, it will be seen that the form of the curve undergoes considerable modification as the temperature is lowered. To apply the reasoning of Smith and Brown to this experiment it may be observed that if the effect of lowering the temperature were to uniformly lower the resistance of the red corpuscles to water laking, the form of Curve *a* would be practically preserved but the whole curve would be moved considerably to the right on the chart. That the inner form of the curve is greatly changed while the whole curve is moved to the right indicates, not only that the general resistance to the laking effect of water is decreased as the temperature decreases, but that some groups of corpuscles are much more affected than are others. I am not prepared to discuss the particular form taken by these modified curves at present. Other experiments charted in the same way have shown internal changes in the curve of the same general nature, but the number of experiments is too few to make any attempt at generalization possible at this time.

Examination of the tables, or better the chart, makes it apparent that the differences between the older results obtained by Hamburger and those here presented are accounted for by a technical departure, that of developing the whole resistance curve, instead of relying on the point of beginning hemolysis for the information sought. It is true that the differences so pronounced in the center of the curve are generally maintained at either end. But at the ends of the series the total quantity of corpuscles of which effective use is made in the test, would be from one-fourth to two or five per cent. of the whole, depending on the closeness with which the end-point is determined, and the percentage difference in hemolysis relied on would be from one-fourth to two or five per cent. These small differences in amount of hemolysis, while recognizable when controlled by the striking differences at the center of the curve, would probably be neglected as within the probable limits of experimental error, if taken alone. If to correct the fault of using too small a number of corpuscles in the regions of beginning hemolysis

a larger absolute quantity of corpuscles were used in the series, the effect would be chiefly to extend the range of hemolysis. This would put the point where laking was first noticeable in a higher concentration of salt solution, but would not increase the absolute amount of laking at the end-point sufficiently to make the method more accurate. At the middle of the series, on the other hand, over forty per cent. of the total number of erythrocytes employed may be affected by a change of two-hundredths of one per cent. of salt in solution or its equivalent in changed corpuscle resistance.

How is the increase in hemolysis with decreasing temperature to be accounted for? Two factors must be considered, the erythrocytes and the saline solution as well as the relation between the two. The relation between erythrocyte and surrounding medium has usually been considered in this connection to be analogous to the relation between a cell with semipermeable membrane, having contents of certain osmotic tension and immersed in a medium of somewhat similar osmotic tension. So long as the osmotic tensions within and without the cell are equal the membrane would be under no strain. After finding no change in the amount of hemolysis with changing temperature Hamburger reasoned that the supposed fact was in harmony with these physical conditions. The fluids within and without the cell would be altered together, in their osmotic tensions, as the temperature changed; there would consequently be no occasion for fluid to pass in either direction. This is certainly correct for the case in which the osmotic tensions within and without the cell are equal. But equality can never be the case under the conditions as at present considered. The intra-corpuscular tension is usually stated to be a little above or below the equivalent of nine-tenths per cent. of sodium chloride solution according to the animal from which the blood is derived. The percentage of salt solution in which the laking is most effectively studied is just above or below five-tenths per cent. sodium chloride, or somewhat more than half of the intra-corpuscular equivalent.

According to the law that osmotic pressure of substances in solution increases directly with the absolute temperature, the absolute increase when the temperature is raised from 5° C. to 35° C. would be to 308/278 of the pressure of the solution with which we

started. With a .50 per cent. salt solution the increase expressed in terms of percentage salt solution would be .056; with a .90 per cent. solution as a starting point the absolute increase expressed in the same terms would be .098. If simply this law were in operation, therefore, an increase in temperature within the limits used should increase the relative concentration of the corpuscular contents as compared with that of the surrounding medium by an amount equivalent to the difference between the above increases. Expressed in terms of salt solution concentration this difference would be .041 per cent. This would, if acting alone, cause an increase in hemolysis with increasing temperature. The difference would be about equal to that which we actually find to occur in the opposite direction.

Other purely physical factors operating in the internal and external fluids are the degree of electrolytic dissociation and the changes in volume. The differences due to these changes would be relatively slight and may be safely disregarded.

Considering the purely physical factors involved has then served to emphasize the value of the difference as experimentally determined. The factor remaining and the one to the properties of which we must look for an explanation of the difference in degree of hemolysis is the cell membrane. The properties of this membrane are almost unknown at present. It has been shown by a number of methods not to be perfectly impermeable to various ions. If this partial permeability were less at lower temperature the tendency for an equilibrium to be established between the contents of the cell and the surrounding medium would be less effective in protecting the cell against the results of sudden osmotic pressure changes in the latter. The membrane can hardly be of the rigid character of the cellular membrane of plant cells. It must rather be of viscous or semi-gelatinous nature kept in place by its lack of solubility in the fluids and substances with which it is in contact. If on cooling, the rigidity of the membrane were increased after the manner of gels in general it would become more friable, less elastic and the quantity of water needed to swell the cell to the breaking point would be less.

It is not worth while to speculate further along this line, how-

ever, especially in view of the probability that the above-mentioned analogy is not a perfect one. When we are considering only physical changes in the surrounding medium it may be entirely pertinent to regard the cell as a definite membranous sack enclosing fluid contents. But when the question is approached from the point of view of the erythrocyte it is much more probable that we have to do with a mass of colloids and electrolytes in more or less close physical and chemical apposition. The conditions in this mass must be very complicated and it would be impossible at the present time to draw reasonable conclusions as to the manner of action of any condition or substance which affects the whole mass.

So far I have commented only on the changes which take place with temperature variation between the limits of a point somewhat above freezing and the normal body temperature. It will have been noted by those who have examined the tables presented that they include some observations at temperatures of from 42° C. to 43° C. At these temperatures I have always found an increase in hemolysis with horse erythrocytes. The hemolysis with rabbits, calf and guinea-pig erythrocytes on the contrary has continued to decrease up to 42° C. to 43° C. Whether the observed differences are constant and represent a definite species character may be determined only by future observations.

SUMMARY.

Decreasing the temperature from 37° C. to 5° C. perceptibly and regularly increases hemolysis in hypotonic sodium chloride and cane sugar solutions, when the erythrocytes of a number of the common mammals are considered. The measurements were carried out with Smith's modification of the method of Hamburger. If following the original method of Hamburger one relies on the point of beginning hemolysis as an index of corpuscle resistance, the facts are not brought out clearly. The effect is in the opposite direction from that which would prevail if the laws governing change of osmotic pressure with change of temperature were the influential factors. The results possibly depend on some change in the permeability or consistence of the erythrocytic protoplasm considered as a semi-permeable membrane.

THE BUTYRIC ACID TEST FOR SYPHILIS IN THE DIAGNOSIS OF METASYPHILITIC AND OTHER NERVOUS DISORDERS.[1]

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General paralysis, although in its clinical aspects a disease that can be frequently diagnosed almost at a glance, is often simulated so closely by other disorders and has so many imitators among the psychoses produced by alcohol, vascular lesions and other causes, that various tests have been devised in recent years for its more certain detection. This paper will be devoted to a consideration of the butyric acid reaction (Noguchi) in relation to the diagnosis of syphilitic affections of the central nervous system, in which class of diseases general paralysis is included.

We have subjected the cerebro-spinal fluid from a series of cases of general paralysis, tabes, cerebral and spinal syphilis and some other mental diseases to this test[2] and at the same time to the complement-fixation test of Wassermann[3] and to cyto-diagnosis according to the method of Ravaut.[4]

[1] Received for publication March 15, 1909.

[2] Noguchi, *Jour. of Exper. Med.*, 1909, xi, 84; *Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vi, 51.

[3] Wassermann and Plaut, *Deutsche med. Woch.*, 1906, xxxii, 1937.

[4] Ravaut, *Ann. de dermat. et de syph.*, 1903, iv, 537.

THE BUTYRIC ACID TEST.

The cerebro-spinal fluid of syphilitic individuals contains, as does the blood serum, a higher content of globulin and especially of euglobulin than normal cerebro-spinal fluid. It is this increase that makes the butyric acid test applicable to the diagnosis of syphilis.

The mode of applying the test is as follows: one or two parts[5] of spinal fluid[6] are mixed with five parts[7] of 10 per cent. butyric acid solution[8] and are heated over a flame to a brief boiling. One part[9] of normal solution of sodium hydroxide is then added quickly to the heated mixture and the whole is boiled once more for a few seconds. The presence of an increased content of protein in the spinal fluid is indicated by the appearance of a granular or flocculent precipitate which gradually settles under a clear supernatant liquid. The intensity of the reaction varies greatly according to the amount of protein which a given specimen contains, but the granular appearance of the precipitate means a positive reaction for syphilis or parasyphilitic affections.

With normal or non-specific specimens there will be a slight opalescence or sometimes a marked turbidity which, however, does not settle out in several hours or even in twenty-four hours.

The test was applied in this manner to all the samples of cerebro-spinal fluid examined and, at the same time, portions of the samples were tested by means of the Wassermann syphilis test which being now established as indicating syphilitic infection in a high percentage of cases with definite histories must be accepted as giving trustworthy results in cases in which definite histories are not obtainable. In some instances the modification of the Wassermann test introduced by one of us[10] (Noguchi) was used in place of the original method.

The cytological examination, which was the second form of control employed, was that of Ravaut, but with one point of difference,

[5] 0.1 or 0.2 c.c. are sufficient and convenient.
[6] Must not contain blood.
[7] 0.5 c.c. for the quantities above specified.
[8] Best in 0.9 per cent. salt solution.
[9] Namely, 0.1 c.c. in this case.
[10] Noguchi, *Jour. of Exper. Med.*, 1909, xi, 392.

as stated, namely, the use of Ehrlich's triacid stain, which was found very satisfactory. In most of the cases two or more such examinations of the cerebro-spinal fluid were conducted at intervals. It is important to note that in no case did the results of the different examinations vary essentially. The rule adopted by us for the cyto-diagnosis was to regard less than ten cells to a field of the microscope given by a one-sixth lens as negative, more than twelve cells as positive, and ten, eleven and twelve cells to such a field as doubtful. The drop employed came within the limits of a one-third inch objective; but experience enables one to make an accurate calculation even when the drop is less uniform in size or has changed by evaporation. The presence of a small number of red corpuscles does not prevent accurate estimation of the white cells. The modification of the cytological method introduced by Alzheimer[11] was employed in a number of instances but without practical advantages.[12]

Since Nonne and Apelt[13] had already observed an increase in the protein fraction, probably consisting of globulin, in the cerebro-spinal fluid of general paralytics, constituting their so-called "phase I," we applied their method to a large number of cases but with very unsatisfactory results. The poor definition obtained between positive and negative readings made the deduction difficult and questionable.

RESULTS IN WHICH THE DIAGNOSIS WAS REASONABLY CERTAIN.

In Table I we have brought together our results in 126 cases whose diagnoses could be made reasonably certain from the symptoms. In a part of the cases the diagnosis was confirmed subsequently by autopsies.

The table brings together the results of the three tests mentioned on several classes of syphilitic affections and on a smaller number of cases of nervous affections in which a syphilitic element could be excluded with a high degree of positiveness. A comparison shows immediately that there is a close correspondence in the results

[11] Alzheimer, *Centralbl. f. Nervenh. und Psychiat.*, 1907, xxx, 449.

[12] The cytological study has been carried out by Dr. Moore at the Pathological Institute of the State Hospitals.

[13] Nonne and Apelt, *Arch. f. Psychiat.*, 1907–8, xliii, 433.

TABLE I.

Results in Cases in Which the Diagnosis Was Reasonably Certain.

Cases.	No. of cases.	Butyric acid reaction.			Wassermann reaction.			Cell count.		
		+	−	±	+	−	±	+	−	±
Syphilis										
Secondary stage (without nervous symptoms)	3	3	0	0	0	3	0	0	3	0
Tertiary stage (without nervous symptoms)	1	1	0	0	0	1	0	0	1	0
Cerebral syphilis	3	3	0	0	1	1	1	3	0	0
Spinal syphilis	3	3	0	0	2	1	0	3	0	0
Hereditary syphilis	10	9	0	1	8	2	0			
Metasyphilis										
general paralysis										
cerebral	43	37	4	2	32	6	5	39	2	2
tabetic	17	17	0	0	12	3	2	16	1	0
Tabes	11	11	0	0	6	4	1	11	0	0
	91	84	4	3	61	21	9	72	7	2
Psychoses										
Arterio-sclerotic	3	1	2	0	1	2	0	1	2	0
Traumatic	2	0	2	0	0	2	0	0	2	0
Senile	1	0	1	0	0	1	0	0	1	0
Epileptic	6	0	6	0	0	5	1	0	6	0
Alcoholic	7	0	6	1	3	3	1	0	6	1
Manic-depressive	2	0	2	0	1	1	0	0	2	0
Dementia precox	11	1	10	0	1	8	2	1	10	0
Imbecility	2	0	2	0	0	2	0	0	2	0
	34	2	31	1	6	24	4	2	31	1

obtained. On closer inspection the results of the butyric acid reaction are found to agree more nearly with those of cytodiagnosis than does the Wassermann test. The explanation for this may reside in the fact that there sometimes occurs in the cerebro-spinal fluid, as in the blood-serum, substances which are antihemolytic and which, therefore, inhibit hemolysis even when all antigen has been omitted from the mixtures. When the attempt is made to eliminate the action of the antihemolysis by diminishing the quantity of cerebro-spinal fluid in the mixtures, a doubtful reaction may result because of too great dilution of the substances acting as antibody.

It is to be regretted that the number of cases of secondary and tertiary stages is so small. The explanation for this fact is to be found in the difficulty of securing the consent of patients not suffer-

ing from nervous affections to submit to lumbar puncture. The results obtained in the four cases are, however, instructive. It will be observed that all four gave the butyric acid reaction and were negative to the Wassermann test and to cytodiagnosis. The explanation of the discrepancy is probably as follows: in active, secondary and tertiary syphilis the increase in globulin of the blood plasma affects also the cerebro-spinal fluid, but the absence of specific lesions of syphilis in the central nervous system determines that the cells are not increased and the antibody on which Wassermann's test depends is not produced *in loco* and does not pass with the fluid in sufficient amount from the blood to give the reaction. Moreover, the discrepancy also indicates that there is no necessary relation between the globulin-content and the Wassermann test. The three cases of cerebral syphilis gave positive results with the butyric acid test and cytodiagnosis but remained doubtful to the Wassermann test.

The most striking results are given by the cases of general paralysis of which more than 90 per cent. reacted positively. The cases of the tabetic forms of general paralysis and of simple tabes all gave positive reactions. The close agreement between the butyric acid reaction and cytodiagnosis is in contradistinction to the discrepancy between the latter and the Wassermann test.

AN ANALYSIS OF THE RESULTS FROM THE STANDPOINT OF SYPHILIS.

In order to study the reactions in relation to metasyphilitic affections, we shall omit those cases with obvious active symptoms of syphilis and disregard all cases in which a reliable history is lacking and the physical examination was insufficient to establish the existence of a previous syphilitic infection. As the majority of the cases comprising our material were derived from an hospital for the insane, only a comparatively small number lend themselves to this consideration. Fifty-nine cases are available for analysis.

Those classed as syphilis + are cases with good histories or unmistakable signs of syphilis or both. Those classed as syphilis — gave intelligent statements, denied the disease and no indication of it could be found.

The results show that cases of general paralysis and tabes with definite histories of a syphilitic infection in early life all yield posi-

TABLE II.

Analysis of the Reactions With Regard to Syphilis.

Cases.	No. of cases.	Butyric acid reaction.			Wassermann reaction.			Cell count.		
		+	—	±	+	—	±	+	—	±
General paralysis and tabes										
Syphilis +	36	34	1	1	26	8	2	36	0	0
Syphilis —	16	12	3	1	10	3	3	11	3	2
Other diseases										
Syphilis +	1	1	0	0	1	0	0	1	0	0
Syphilis —	12	1	11	0	3	8	1	1	11	0

tive reactions to all the tests in a very high percentage, amounting to 100 per cent. for cytodiagnosis, 94 per cent. for the butyric acid test and 72 per cent. for the Wassermann test. It is noteworthy, however, that the same class of diseases affecting individuals without previous histories of a syphilitic infection also give a high, though somewhat lower percentage than the first group. For the butyric acid test, which was the highest, the percentage was 75, for the cell count it was 60 and for the Wassermann test 62.5.

The clinical features of these two groups of cases, namely, the one with definite syphilitic histories and the other without, were not different in any way; moreover, the severity of the symptoms does not always run parallel with the intensity of the reactions. There existed, however, a rough parallelism between the increase of cells and the strength of the butyric acid reaction.

With the cases which were neither paralytic nor tabetic, the results were entirely different. A case of dementia precox with a reliable history of a previous syphilitic infection gave positive reaction to all the tests.

Twelve cases without histories of syphilitic infection were examined with the result that one gave positive reaction to all the tests and two others reacted positively only to the Wassermann, but not to the other two tests. All cases of dementia precox constituting this group reacted negatively to all the tests.

It may be recalled here that Raviart, Breton and Petit[14] obtained about 27 per cent. of positive reactions with the Wassermann test using the blood of cases suffering from dementia precox.

[14] Raviart, Breton and Petit, *Rev. de Méd.*, 1908, xxviii, 840.

THE BUTYRIC ACID TEST AND POST-MORTEM FINDINGS.

In all, seventeen cases of the series have come to autopsy. Fifteen had given positive findings with the butyric acid test, fourteen being diagnosed as general paralysis and one as cerebral syphilis. Two had given negative results with the acid. The autopsy findings and the results of the test agreed in every case.

In a number of cases the results of the examination of the cerebro-spinal fluid has been the means of establishing an otherwise doubtful diagnosis or of correcting an erroneous one. This is true of cases giving negative as well as positive reactions. In how far this result will be more generally applicable to diagnosis, only a wide series of observations can decide. The outlook seems extremely hopeful.

THE BUTYRIC REACTION IN GENERAL DISEASES.

It would appear that the butyric acid reaction will come to serve a useful purpose in psychiatric diagnosis. This is the opinion of Mott[15] also, who has already expressed his opinion. It is, however, necessary that the limitation of its applicability should be precisely defined by subjecting the cerebro-spinal fluid from other general diseases to the test. Such a wide examination can alone determine whether the reaction is specific for syphilitic lesions, which at the outset seemed doubtful, or whether the changes in the globulin concentration of the cerebro-spinal fluid occurred in many other diseases than syphilis. It could be predicted, of course, that in all conditions of acute exudative inflammations of the cerebro-spinal meninges, the reaction would be obtained. The change in the character of the fluid and the increase in blood proteins predetermined this result. Table III shows that in all acute inflammatory conditions of the cerebro-spinal meninges the reaction is positive. There is, however, no difficulty whatever in making a diagnosis in this class of cases and they do not, therefore, come into account. The precipitate given with butyric acid is very heavy as would be expected. Even with tubercular meningitis the symptoms and other criteria easily differentiate that condition from syphilitic or metasyphilitic ones. The cases of hydrocephalus are a little more con-

[15] Mott, *British Med. Jour.*, 1909, i, 454.

fusing, but as only two came under observation and one gave the Wassermann test also, the existence of congenital syphilis should be thought of. The general diseases, among which are typhoid fever and pneumonia, with which meningeal implication may be absent are not attended by changes in the cerebro-spinal fluid that lead to the butyric acid reaction.

TABLE III.

The Butyric Acid Reaction in General Diseases.

Cases.	No. of cases.	Butyric acid reaction.			Wassermann reaction.		
		+	−	±	+	−	±
Diseases of the meninges							
Epidemic cerebro-spinal meningitis	14	14	0	0	0	14	0
Pneumococcal meningitis	6	6	0	0	0	6	0
Influenzal "	1	1	0	0	0	1	0
Tubercular "	30	30	0	0	0	30	0
Hydrocephalus externus	2	2	0	0	1	1	0
	53	53	0	0	1	52	0
Diseases without meningeal involvement							
Typhoid fever	1	0	1	0	0	1	0
Pneumonia	4	0	4	0	0	4	0
Pulmonary tuberculosis	1	0	1	0	0	1	0
Entero-colitis	2	0	2	0	0	2	0
Rachitis	1	0	1	0	0	1	0
Uremia	2	0	2	0	0	2	0
Septicemia	1	0	1	0	0	1	0
Miscellaneous without nervous involvement	12	0	11	1	0	10	0 2
	24	0	23	1	0	22	2

On the basis of these tests, we consider that the butyric acid reaction suffices to distinguish normal from pathological cerebro-spinal fluid and especially from that fluid which has been altered through an increase in its protein constituent. We would, therefore, point out that by its means it may be possible to determine the limits of some ill-defined inflammatory conditions of the meninges, such as, for example, the so-called meningitis serosa, in which condition microörganisms have not yet been demonstrated satisfactorily and the cellular exudations are absent, and yet abnormal serous exudations seem to occur. If these exudations contain an excess of protein constituents as compared with the normal transudate the differ-

ence should be appreciable by this test. Moreover, whether this increase is due to or attended by syphilis could be determined by the Wassermann test or the modification of it recommended by one of us (Noguchi).

We believe, moreover, that in tubercular meningitis the butyric acid reaction will always be positive. In a suspected case, therefore, in which no tubercle bacilli have been found and the tuberculin reaction is unconvincing, this test, if negative, would have diagnostic value. We have applied the test to a small number of doubtful cases of suspected tubercular meningitis. Those giving negative results subsequently proved not to be tubercular.

SUMMARY.

1. In the secondary and tertiary stages of syphilis without direct involvement of the nervous system the cerebro-spinal fluid yielded the butyric acid reaction of feeble intensity. The fluid under these conditions gave neither a positive cytodiagnosis nor the Wassermann test for syphilis.

2. The cerebro-spinal fluid of cases of hereditary syphilis showed a positive butyric reaction in about 90 per cent. and a positive Wassermann test in about 80 per cent. of the cases examined.

3. The cerebro-spinal fluid of cases of cerebral and spinal syphilis yielded the butyric acid reaction and the cytodiagnosis in every instance (100 per cent.) and gave the Wassermann reaction in from 50 to 75 per cent. of those examined.

4. The cerebro-spinal fluid from cases of general paralysis gave positive butyric acid reactions in 90 per cent., positive cell counts in 91 per cent., and positive Wassermann test in 73 per cent. of those examined.

5. The cerebro-spinal fluid from cases of tabes dorsalis gave positive butyric acid reactions and cell counts in 100 per cent. and positive Wassermann test in 53 per cent. of those examined.

6. The cerebro-spinal fluid from other forms of psychosis in which a syphilitic history was not obtained gave positive butyric acid reactions and cell count in 2.8 per cent. and Wassermann test in 13 per cent. of those examined.

7. The cerebro-spinal fluid from cases of acute inflammatory diseases of the meninges always gave a flocculent precipitate with the butyric acid reaction but never gave the Wassermann test.

8. The cerebro-spinal fluid from persons suffering from typhoid fever, pneumonia, pulmonary tuberculosis, etc., in which the meninges are not inflamed, gave neither the butyric acid nor the Wassermann test.

9. We think it justifiable to conclude that the butyric acid reaction affords a ready means of distinguishing normal from pathological cerebro-spinal fluid and will prove to be useful in routine clinical practice, especially in detecting syphilitic disease and in confirming or setting aside certain doubtful diagnoses of syphilitic or metasyphilitic lesions of the central nervous system. The reaction should commend itself as a valuable addition to the Wassermann test, the results of which it is capable of confirming and extending.

STANDARDIZATION OF THE ANTIMENINGITIS SERUM.[1]

By JAMES W. JOBLING, M.D.

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The difficulties encountered in standardizing the bactericidal type of immune sera are great and have not yet been successfully mastered. They are especially great in the case of the antimeningitis serum in which bacteriolytic power cannot be utilized as a measure of strength and protective and curative effects, and the experimental Diplococcus intracellularis infections in lower animals are also unavailable. The diplococcus of meningitis is not brought to bacteriolysis by the immune serum through amboceptor and complement, and the high variability in pathogenic power of its cultures makes the results of experimental inoculations very untrustworthy. The fatal dose of cultures and of autolyzed toxines fluctuate within wide limits for animals (guinea-pigs) of the same age and weight. The attempt to estimate quantitatively the strength of the antiserum by means of complement deviation cannot be said to have been successful,[2] and in our hands has not yielded accurate or uniform results.

The study of the changes which take place, under the influence of the antiserum, in inoculated animals and in human beings suffering from epidemic cerebro-spinal meningitis indicates unmistakably that the antiserum possesses power to destroy directly the diplococcus, to neutralize a certain amount of the endotoxin yielded by the organism, and above all to bring about an increased phagocytic inclusion and digestion of the diplococcus. The first convincing proof of the manner of action of the antiserum was supplied by the studies of Flexner[3] carried out on monkeys in which an experi-

[1] Received for publication March 1, 1909.

[2] Kolle and Wassermann, *Deut. med. Woch.*, 1907, xxxii, 609.

[3] Flexner and Jobling, *Jour. of Exper. Med.*, 1908, x, 141.

mental cerebro-spinal meningitis had been produced by direct inoculation of the spinal membranes with cultures of Diplococcus intracellularis. In brief, Flexner found that in monkeys receiving a lethal dose of the culture an inflammation quickly set in and the diplococci appeared partly free in the fluid exudate and partly within leucocytes quite as in the natural disease in man. In the animals not injected with the antiserum the conditions remained essentially unaltered until death, except that some multiplication of the diplococci sometimes seemed to take place. In the animals receiving the antiserum, the diplococci diminished in number, tended to be entirely taken up by leucocytes, and to suffer rapid degeneration within the latter. These findings led to the view that part of the beneficial action of the antiserum was due to the increased phagocytosis caused by it, a conclusion which has since been verified in the study of many cases of epidemic meningitis in human beings which were treated with the antiserum.[4]

It should be mentioned that Jochmann[5] and Kolle and Wassermann[6] reported uniform results of tests with the antiserum in protecting small animals—mice or guinea-pigs—from the living cultures and thought that use could be made of this method of standardization. But a critical examination of their figures will soon dispel all confidence in its usefulness although they indicate a greater power of the immune serum to protect animals than is possessed by normal serum. A similar conclusion was independently reached by Flexner.[7]

Jochmann,[8] on the other hand, pointed out first that the antiserum possessed greater power of stimulating phagocytosis *in vitro* than normal serum, thus bringing it into line with other immune sera in respect to opsonic properties, but he did not attempt to determine the limits of the opsonic power.

It is difficult to express an opinion on the highly divergent results of Ruppel[9] who claims to have secured a strain of the diplococcus

[4] Flexner and Jobling, *loc. cit.*
[5] Jochmann, *Deut. med. Woch.*, 1907, xxxii, 788.
[6] *Loc. cit.*
[7] Flexner, *Jour. of Exper. Med.*, 1907, ix, 105, 142.
[8] *Loc. cit.*
[9] Ruppel, *Deut. med. Woch.*, 1906, xxxii, 1366.

that is uniformly fatal to mice in doses of 1 cubic centimeter of a 1 to 1,000,000 dilution, and employs this culture in standardizing an antiserum. I believe that in the present state of our knowledge of Diplococcus intracellularis, we are justified in viewing Ruppel's claims with suspicion.

Kraus and Doerr[10] are the latest writers to recommend the use of soluble products of the diplococcus, prepared either by Flexner's toluol method[11] or by extraction with N/10 sodium hydrate, for standardization and determination of anti-endotoxic power of the antiserum. The figures given by them do not inspire confidence in the method.

My own experiments with extracts prepared by autolysis and by alkali extraction have been entirely disappointing. In the effort to secure more uniform results the proteids were precipitated from the extracts with alcohol, dried, redissolved in sodium chloride solution, reprecipitated and dried. The resulting powder readily dissolved in water and salt solution but acted irregularly whether administered by subcutaneous intraperitoneal or intracardiac injection in guinea-pigs weighing from 200 to 225 grams, which is the most uniformly susceptible size. The fatal dose varied from 0.001 to 0.01 gram. I am convinced that nothing is to be achieved by this method of standardization.

The methods which have been worked out recently for determining quantitatively the opsonins in serum offer more hope in securing a unit of value for standardization, especially since it has been established that the opsonic power of the antiserum is an important factor in respect to the therapeutic effects. Neufeld[12] is the first person to make quantitative estimations of the opsonic strength of antimeningitis sera and to publish his results. He tested several different samples of antimeningitis sera to determine the greatest dilutions at which they would still cause phagocytosis. The limits of variation were considerable: some samples were active in dilutions of 0.0002 to 0.0005 per cubic centimeter and others only on 0.01 per cubic centimeter.

[10] Kraus and Doerr, *Wien. klin. Woch.*, 1908, xxi, 12.
[11] Flexner, *Jour. of Exper. Med.*, 1907, ix, 105.
[12] Neufeld, *Med. Klin.*, 1908, iv, 1158.

Neufeld is strongly of the opinion that the favorable action of the antimeningitis serum depends largely on its bacteriolytic effect and hence he believes that a measure of the opsonins would also be a measure of its activity and would suffice for standardization. He also admits other effects of the serum including an anti-endotoxic property but he regards them as subordinate to the opsonic property.

I used, at first, in the quantitative study of the opsonins of our antiserum the method recommended by Neufeld, but later I added to it the direct method of counting the included diplococci by a modified Leishman's method. I shall describe the two methods employed in brief and then state the results obtained.

Neufeld's Method.—The leucocytes are obtained from the peritoneal cavity of guinea-pigs by injecting aleuronat one day and collecting the exudate the next day. The cells are washed four times in 0.9 per cent. salt solution. After the last washing they are suspended in such a quantity of the saline solution as to equal in opacity a 0.3 per cent. lecithin emulsion in normal saline.

The emulsion of diplococcus is made by adding one cubic centimeter of an equal mixture of bouillon and salt solution to each slanted tube of twenty-four hours' growth of the diplococcus on beef-infusion-glucose agar. This emulsion is quite thick but it is desirable that it should be.

Not every culture of diplococcus intracellularis is suitable for the test. Neufeld laid great stress on the proper selection of the culture and my experience agrees with his. I examined and discarded numerous cultures as being unsuitable either for the reason that they were too readily digested by the leucocytes, or were not readily subject to phagocytic inclusion. Culture No. 720, which was the one selected, fulfilled the conditions very well. As illustrating the great variation in phagocytosis I might mention that certain antisera caused considerable phagocytosis of this culture in dilutions of 1 to 5,000, while with other cultures a corresponding amount of phagocytosis required a dilution of 1 to 500. Culture No. 720 had been under artificial cultivation for more than a year. But the factor of artificial cultivation is not highly important, as I found that certain recently isolated strains were more easily phagocyted than some strains grown in the laboratory for three years.

The tests are conducted in test-tubes of about twelve millimeters in diameter and five centimeters in length. To each tube are added two drops of the diluted antiserum, one drop of emulsion of diplococcus and two drops of suspension of leucocytes. The tubes are incubated at 37° C. for one and one-half hours. At the expiration of the incubation the leucocytes have settled to the bottom of the tubes so that by careful manipulation the supernatant fluid can be poured off, leaving the leucocytes adherent to the bottom of the tube. The spreads are made from the mass of leucocytes and diplococci by placing a platinum loopful on a slide and spreading it with the inclined edge of a second slide. After drying in the air, the spread is fixed for one minute in methyl alcohol and then stained in a 1 to 10 dilution of Manson's methylene blue solution. The control tube contains salt solution instead of antiserum. Where many tests are being carried on at one time a control tube is made for each six or eight of the series. The serum tested ranged in age from ten days to fifteen months. When the sera were less than fifteen days old they were heated at 60° C. for thirty minutes before testing.

The readings are based on the gross appearance of the spreads without counting the phagocyted diplococci. The spreads made from the tubes containing the higher concentrations of the antiserum showed that very marked phagocytosis had occurred. Many of the leucocytes were completely filled with diplococci; the control spreads would sometimes show leucocytes containing two or three diplococci. In dilutions of the serum at 1 to 1,000 and 1 to 2,000 no difficulty was experienced in interpreting the appearance since the degree of phagocytosis greatly exceeds the controls. When the dilutions exceeded 1 to 2,000 the decision was sometimes less readily made. Whenever doubt existed the tests were repeated several times so as to avoid the experimental error as far as possible.

Modified Leishman[13] *Method.*—Feeling that the figures obtained by the Neufeld method were subject to some difference in interpretation, all the antisera were tested as follows: the leucocytes were derived from the circulating blood of the dog, the emulsion of the diplococcus was prepared much thinner, and capillary tubes were used for the mixture to be incubated, after the manner recom-

[13] Leishman, *Brit. Med. Jour.*, 1902, i, 73.

mended by Leishman. The included cocci were counted and the greatest dilution yielding a higher count than the saline control was taken as representing the ultimate strength of the antiserum.

The figures yielded by the two methods are in remarkable agreement so that it will be necessary to give only one table showing the general results obtained. In practice, the second or Leishman method is somewhat more convenient.[14]

The two methods were applied to the antisera obtained by mixing the sera from the three horses used for immunization as is done in preparing the antiserum for distribution. The table gives the numbers of the sera tested, the numbers of the horses and the age of the antisera at the time of the testing.

TABLE I.

Opsonic Strength of the Mixed Antisera.

Number of Serum.	Horses Used.	Age of Serum.	Greatest Dilution.
7	Nos. 1 and 2	15 months	1– 800
12	Nos. 1 and 2	13½ "	1– 2,000
17	Nos. 1 and 2	10 "	1– 2,000
19	Nos. 1 and 2	9 "	1– 5,000
20	Nos. 1, 2 and 3	8½ "	1– 2,000
21	Nos. 1, 2 and 3	8 "	1– 2,000
22	Nos. 1, 2 and 3	7½ "	1– 2,000
23	Nos. 1, 2 and 3	7⅓ "	1– 3,000
24	Nos. 2 and 3	7 "	1– 1,000
25	Nos. 2 and 3	6½ "	1– 1,000
26	Nos. 1 and 2	4 "	1– 1,000
27	Nos. 1, 2 and 3	3½ "	1– 800
28	Nos. 1, 2 and 3	3 "	1– 2,000
29	Nos. 1, 2 and 3	2½ "	1– 5,000
30	Nos. 1, 2 and 3	2 "	1– 5,000
31	Nos. 1, 2 and 3	1½ "	1– 8,000
32	Nos. 1, 2 and 3	15 days	1–10,000
Normal horse serum.		30 and 60 days	1– 80

[14] I am indebted to Dr. R. V. Lamar for a slight modification of the methods which has led to more accurate results. Owing to the readiness with which the diplococcus is dissolved in the leucocytes, it is important to leave them in contact for the briefest possible period. By incubating the serum dilution and diplococci at 37° C. for one hour, before adding the leucocytes, and the mixture of the three components for an additional thirty minutes, maximum phagocytosis is secured and the diplococci are not so acted upon as to lose their staining power.

The variations shown in the table may at first sight appear contradictory, but on analysis they will prove not to be so. In the beginning of the work on the antimeningitis serum, only one horse was used in the preparation of the serum and it was not until several months later that a second horse was subjected to immunization. Sera Nos. 7, 12, 17 and 19 were prepared from a mixture of the sera of horses Nos. 1 and 2. The power rose in this mixed serum from 1 to 800 to 1 to 5,000 in about six months time. Somewhat later a third horse was added, and serum No. 20 is the first lot containing serum from horse No. 3.

The drop in opsonic activity has been attributed to the effect of serum from horse No. 3 that was of low strength. Gradually the strength of the mixture rose to 1 to 3,000 (sample No. 23). Shortly after the preparation of sample No. 23, the horse No. 1 developed fever which continued for several days so that the horse was not bled the next regular time. Hence serum (sample No. 24) was composed of sera from horses Nos. 2 and 3 which had been treated a shorter time than horse No. 1. The drop in opsonic activity is marked, as the limit of the next two samples was only 1 to 1,000. After serum No. 25 was prepared the horses were sent to the farm for the summer—from June until September, during which period they received no inoculation. Samples Nos. 26 and 27 show the falling off due to the summer's rest. Inoculations were resumed and a progressive increase in opsonic strength produced in the combined sera, the highest titre being reached in sample No. 32 in which the limit of dilution was 1 to 10,000. The opsonic value of normal horse serum is low and in the sample recorded was 1 to 80.

TABLE II.

Opsonic Strength of the Separate Antisera.

No. of Serum.	Horse No. 1	Horse No. 2.	Horse No. 3.
No. 26, Sept. 19, '08	1– 2,000	1–1,000	1– 200
No. 28, Oct. 16, '08	1– 8,000	1–2,000	1–1,000
No. 29, Oct. 30, '08	1–10,000	1–2,000	1–2,000
No. 32, Jan. 7, '09	1–20,000	1–5,000	1–2,000

Beginning September, 1908, the sera from the several horses were tested separately several times. Table II gives the results of four tests of each horse and exhibits clearly the effects of inoculations

continued over a long period of time. In September, 1908, Horse No. 1 had been under treatment for two years; horse No. 2 for fourteen months; and horse No. 3 for eight months.

The serum sample of September 9 was drawn before inoculation was re-commenced after the summer's rest. Horse No. 3 has always given a smaller yield of serum for the amount of blood withdrawn than either horse No. 1 or No. 2. Horse No. 1 yields the greatest volume of serum of the three horses. Hence in the mixtures, the diluting effects of the sera of horses Nos. 2 and 3 are less than would have been the case had the yield of serum been approximately equal.

CONCLUSIONS.

The high variability in infectivity of Diplococcus intracellularis makes it impracticable to standardize the antimeningitis serum on the basis of the virulence of the diplococcus.

The irregularity of reaction of small animals to the poison or endotoxin of Diplococcus intracellularis makes it impracticable to standardize the antimeningitis serum on the basis of endotoxic value.

The want of uniformity in the complement-binding power of the antimeningitis serum, and the absence of established relation between complement-binding power and therapeutic activity make the employment of this method of standardization impracticable.

The part taken by specific opsonins in promoting recovery from infection with Diplococcus intracellularis suggests their employment as a measure of the therapeutic activity of the antiserum. Methods of quantitative estimation of opsonic content of the antimeningitis serum being available, it would seem advantageous to adopt for the present as a standard of value a definite and suitable strength in opsonins of the antimeningitis serum.

As a definite and suitable standard of strength a minimum dilution activity of a 1 to 5,000 dilution of the antiserum is proposed.

Since the immune opsonins of the antimeningitis serum appear to be highly durable under proper conditions of refrigeration of the antiserum, the test proposed will be applicable when the antimeningitis serum shall have become an article of commerce.

CONTINUOUS RESPIRATION WITHOUT RESPIRATORY MOVEMENTS.[1]

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The object of the function of respiration is to supply the animal with oxygen and to remove carbon dioxide. To attain this object the vertebrates are provided with a complicated mechanism of which the respiratory movements are an essential feature. The respiration appears as a continuous chain of rhythmically recurring cycles, each cycle consisting of two antagonistic movements, one which carries air into the body and the other which assists its removal from the body. When the muscular activity of the body is eliminated by one cause or other and the exchange of the gases is carried on by so-called artificial respiration, again the respiration is rhythmically discontinuous and each cycle is composed of the two antagonistic movements: the inflow of air is carried on rhythmically by some external mechanism, while the return of the air is accomplished during the intermission by the elastic forces of the body. The rhythmic antagonistic movements seem thus to be inseparable from the function of respiration.

In studying recently the nature of the mechanism of the respiration in the presence of a double pneumothorax, while the animal is breathing compressed air by the Brauer method of overpressure, we discovered the fact that under certain conditions respiration can be carried on by continuous inflation of the lungs, and without any normal or artificial rhythmical respiratory movements whatever. This observation was verified by many experiments and we will describe here briefly the essential features of the experiment.

A longitudinal slit is made in the trachea of an anesthetized dog or rabbit and a glass tube introduced down to the tracheal bifurca-

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tion. The protruding end of the tube is then connected with a pressure bottle by means of a *T*-tube, the opening of the free branch of which is regulated by a screw clamp. The air which streams from the bottle under pressure partly escapes through the free branch of the *T*-tube and partly enters the trachea and reaches the bifurcation from which it returns through the space between tracheal wall and tube and escapes through the slit in the trachea and through mouth and nose. It is essential that the tube should fill out two-thirds of the lumen of the trachea, that the slit in the trachea be not too short and that the pressure of the air which enters the *T*-tube should amount to about fifteen to twenty millimeters of mercury. The pressure within the trachea is of course much lower than that. In the connection between the trachea and the pressure bottle are interpolated a manometer, an ether bottle and a bottle with Ringer's solution to keep the mucous membrane of the trachea moist. The essential point of the arrangement is that air is reaching the bifurcation under pressure and returns through another path than that through which it entered. When the air is thus circulating through the trachea the diaphragm descends, the thorax becomes moderately distended and the respiration mostly becomes very slow. The heart beats also frequently become dangerously slow. This danger, however, is easily obviated by an intravenous injection of one milligram of atropin; in a few seconds the pulse becomes frequent and remains so for many hours. The animal may receive now an intravenous injection of curare sufficient to completely abolish any spontaneous or reflex movements; its life is as safe as under regular artificial respiration. When the anterior thoracic wall is removed, the distended lungs are seen to be immobile while the heart continues to beat with a regular rhythm. If the above described arrangement is carried out properly the lungs retain their pink color, the heart continues to beat regularly and efficiently for many hours and the blood-pressure shows but little variation.

We have observed animals four hours and longer under these normal conditions. If the glass tube within the trachea is a little too wide or too narrow in relation to the lumen of the trachea the lungs acquire easily a slightly cyanotic appearance. But then a disconnection of the tube from the pressure bottle for two

seconds, which means a momentary collapse of the lungs, restores immediately the pink color of the lungs and a repetition of this procedure once every three or four minutes is sufficient to maintain the life of the animal in a satisfactory fashion for many hours, although under these circumstances the blood-pressure is subject to frequent variations.

In another method, the tube which conveys the air to the lungs is short—a regular tracheotomy tube—and is tied in firmly in the upper part of the trachea, while another narrower tube is inserted into the trachea through a narrow opening made at a lower place. This tube reaches the bifurcation and serves for the removal of the air. This method also was found to do satisfactory service. In a third method, a long O'Dwyer tube, bent at right angles, was introduced through the mouth and inserted into the larynx. Through this tube a catheter was pushed into the trachea until it reached the bifurcation. Both tubes were then connected with the pressure bottle in such a manner as to let the air enter through the O'Dwyer tube and escape through the catheter. This arrangement, however, has failed as yet to give uniform results. The method, however, is surely capable of improvement and it is probable that it will finally give satisfactory results.

If the air is made to enter the lungs through a short tracheal tube firmly tied into the trachea, the curarized animals die in a very short time from asphyxia. With this method, the spontaneous respirations of the animals are apparently indispensable for the maintenance of their life. The result is not perceptibly better even if the firmly tied-in tube reaches the bifurcation. The difficulty of this method consists mainly in the fact that the removal of the carbon dioxide has to take place against the stream of the air within the tube; while in our method the removal of the carbon dioxide is rather assisted by this stream of air.

The following three points are the essential factors in the success of our method: (1) The lungs are kept in continuous inspiratory state of distension which facilitates the exchange of the gases. (2) The fresh air reaches the lowest part of the trachea. (3) The air escapes by another path (although also through the trachea) than by the one it enters. Under these conditions the supply of oxygen

and removal of carbon dioxide take place apparently in physiological fashion without the aid of any rhythmic antagonistic movements.

Besides the direct physiological bearing of our experiments on the function of respiration the method is destined to be of methodical service in other physiological investigations, for instance in the study of the heart actions where the movement of the lungs is a disturbing factor. This method might in a certain way offer some advantages over the known methods of Langendorff and of Bock-Hering. Furthermore the method promises to be of practical service in various directions. We shall not omit to refer to two statements in the literature which can be considered as forerunners of our method. In the first place, there is the statement[2] that Hook in 1667 maintained the life of a dog for an hour by continuous inflation of the lungs previously punctured at various places. In the second place, we have to mention Nagel's[3] communication according to which the life of curarized pigeons were maintained by sending a continuous stream of air through the humerus which in birds is connected with the air sacs. In this case the air escaped through the trachea. In both instances the air escaped through the paths opposite to those through which it entered. In our method the air enters and escapes through the trachea, although through the separate paths within it.

[2] Rosenthal, Hermann's Handbuch der Physiologie, 1882, iv, 238.
[3] Nagel, *Centralbl. f. Physiol.*, 1900, xiv, 238.